PHYSIOLOGICAL AND BIOCHEMICAL CHANGES DURING GROWTH AND DEVELOPMENT IN THE SHRIMP *METAPENAEUS ENSIS*

by

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Thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Philosophy

at

The Chinese University of Hong Kong

June, 1987

Marine Science Laboratory

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ABSTRACT

Physiological and biochemical changes during growth and development in the shrimp _Metapenaeus ensis_

This study quantified oxygen consumption, ammonia excretion, and the biochemical composition in the egg, larvae, and juvenile of _Metapenaeus ensis_. The oxygen:nitrogen ratios (O:N) were calculated from these data. The effect of a 24 hour starvation period on metabolic activity was studied in the larval and postlarval stages. The effect of a 3 day starvation period on metabolic activity and biochemical composition was studied in the juvenile, as well as the effect of a 5 C temperature difference on changes in metabolic activity during starvation.

The lipid and carbohydrate fractions both decreased, as the larvae developed to postlarvae. Between the protozoea III larval stage and the 3 day old postlarvae, an increasing weight specific rate of oxygen consumption suggested an increasing energy demand. _Metapenaeus ensis_ switches from a planktonic to a benthic habitat when the postlarvae are about 5 days old. There was a reduction in oxygen consumption in the 9 day old postlarva, when compared to the 3 day old postlarva, associated with the shift to a benthic existence.

The O:N ratio declined between the egg and the nauplius V/VI, coupled with a depletion of lipid and carbohydrate, indicating a greater utilization of protein in the nauplius. The O:N ratio increased between the nauplius V/VI stage and the 9 day old postlarva, under fed conditions, and was the same in the juvenile as the 9 day old postlarva. This indicates a greater utilization of dietary carbohydrate or lipid in the later developmental stages.

Size was found to have a negligible effect on metabolic rate within a 17-177mg wet weight size range in juveniles. The larvae, however, did have a much higher weight specific metabolic activity than the juveniles.

In the larval and postlarval stages, the effect of a 24 hour starvation period was a reduction in oxygen consumption. Starvation had little effect on ammonia excretion. The O:N ratio after 24 hours of starvation was lower.

In the juveniles, the carbohydrate and lipid fractions were both depleted after one day of starvation. This depletion, coupled with the continued increase in ammonia excretion over a 3 day starvation period and a decreased O:N ratio, suggests initial utilization of carbohydrates and lipids, followed by a carbohydrate-lipid-protein mixture. A 5 C temperature difference had little effect on changes in metabolic activity during starvation.

ACKNOWLEDGEMENTS

This is the only section of my thesis in which I have more to write than space allows. During my stay in Hong Kong I have received much assistance and encouragement, not only directly related to my research but also to my acceptance and enjoyment of life in Hong Kong. The latter was of course crucial to the successful completion of my studies.

I would like to thank my thesis committee members, Drs. Ka Hou Chu, Norman Y.S. Woo, and K.W. Chiu. I especially thank Dr. Chu, my academic advisor, for his guidance and help with my research, and for tirelessly and repeatedly reading this manuscript. I would also like to thank Dr. Woo for his invaluable criticism.

The staff of the Marine Science laboratory have all been an enormous help. I thank Mr. Y.C Tam and W.K. Chu for their help in purchasing shrimp spawners, raising the larvae, and countless other deeds; Kenneth Au for his constant assistance, especially in teaching me how to prepare figures for this thesis; and Mr. K. Ngan and Mr. M.K. Cheung for their assistance and invariably cheerful attitudes. I would like to thank my fellow students, Anthony Chung for writing his thesis just before I did and so good naturedly answering my never ending stream of questions, and Lam Hon Ming for his kind voluntary assistance in preparing slides. The technical staff in the

Biology Department also provided assistance: instruction on how to freeze dry samples and how to develop film. I thank Mr. Edison Lee for supplying the juvenile shrimp for my research.

Two special friends deserve more thanks than I can express. Juliette Chen and Chris McNally received the brunt of my bad humor and took it all very good naturedly. Without Juliette's company and food I would have been a skinny basketcase.

Finally, I thank the Hong Kong Department of Education, and the Ministry of Education and Culture in the Bahamas for nominating me, for the two year scholarship under the Commonwealth Scholarship and Fellowship Plan. This research and all my memorable experiences in Hong Kong were made possible by this award.

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CHAPTER I

General Introduction

Penaeid shrimp biology has been studied since the 1930's. This research has led to a tremendous increase in our understanding of the shrimp life cycle, and to the establishment and rapid growth of a shrimp aquaculture industry (See Wickens, 1986 for a review). The species Penaeus japonicus was first successfully cultured in Japan by M. Hudinaga in 1942 (Hudinaga, 1942, in Shigeno, 1969). Hudinaga started research on shrimp culture in 1933 and has the honor of being known as the father of shrimp culture. With the growth of commercial shrimp culture, most research has taken the traditional dietary studies approach (see New, 1976 for a review). Basic physiological studies which quantify metabolic functions (such as oxygen consumption and ammonia excretion) were initially ignored. Due to the success of the mariculture industry, research has been devoted to important culture species and increasingly deals with physiological aspects. Mariculturally important species which have received attention include Homarus americanus (Capuzzo and Lancaster, 1979; Anger et al., 1985; Sasaki et al., 1986), Macrobrachium rosenbergii (Clifford and Brick, 1978, 1979, 1983), and Penaeus esculentus (Dall, 1986; Dall and Smith, 1986). It is important to understand metabolic activity and type of energy utilization in order to obtain favorable survival and growth results with efficient food utilization.

A weakness in shrimp culture lies in the production of gravid females in captivity to produce fertilized eggs. Culture operations must often rely on wild caught gravid females to produce larvae. Farming operations are often limited by the availability of postlarvae to stock grow out facilities. It is therefore important to obtain the best survival possible in the hatchery rearing of freshly hatched nauplii to the postlarval stage. A better understanding of the physiology and biochemical composition of the larval, postlarval, and juvenile stages, and any changes that occur during this transitional period, will contribute to this aim.

This dissertation deals with physiological aspects and biochemical composition of the shrimp Metapenaeus ensis. Two physiological parameters, oxygen consumption and ammonia excretion, have been quantified, the resulting O:N ratios calculated, and the biochemical composition determined through larval and postlarval development and in the juvenile.

Oxygen consumption is the most frequently used parameter in crustacean respiratory physiology. The rate of oxygen consumption is a good indicator of metabolic rate and depends on many intrinsic and extrinsic factors. In the study of metabolism the ratio of oxygen consumed to nitrogen excreted (the O:N rati. is an important physiological index (Conover, 1968). The usefulness of this ratio in physiological studies of marine organisms was first demonstrated by Harris (1959) who studied natural plankton populations. The O:N ratio is a good indicator of the state of general metabolism, reflecting the level of activity of oxidative and protein metabolism. Changes in the O:N ratio may be accompanied by changes in biochemical composition and in substrate utilized for the energy source. This is the subject of this dissertation.

The species *Metapenaeus ensis* (de Haan), known in Hong Kong as the sand shrimp, is a popular species cultured in Hong Kong and the neighboring regions of China. During its larval development, *Metapenaeus ensis*, like other penaeid shrimps, experiences a complicated series of changes in morphology and feeding behavior (Tseng *et al*., 1979; Chu and Shing, 1986). The non-feeding nauplius becomes a filter feeding, planktonic herbivore and then an encounter or raptorial omnivore. During the postlarval stage the planktonic animal settles to the bottom becoming a benthic omnivore. With these changes in morphology and behavior, physiological and biochemical changes might also be expected.

The specific objectives of this research are:

1. To determine the oxygen consumption rate, ammonia excretion rate, and biochemical composition during ontogenetic development and in the juvenile of

Metapenaeus ensis.

2. To examine the effect of starvation on oxygen consumption and ammonia excretion in *M. ensis* from the protozoea stage to the juvenile, and concurrent changes in biochemical composition in the juvenile.
3. To compare the oxygen consumption and ammonia excretion at two temperatures in fed and starved juveniles.
4. To calculate oxygen:nitrogen ratios which may suggest biochemical substrate utilization.

The following chapter reviews relevant literature. This research is then presented in two sections, the division being based on the stage of development.

Chapter III covers the stages from egg through postlarva. Chapter IV deals with the juvenile. General conclusions follow in chapter V.

CHAPTER II

Literature Review

Aspects of crustacean metabolism have been extensively covered in the literature. Respiration has been reviewed by Wolvekamp and Waterman (1960) and excretion reviewed by Parry (1960). More recently oxygen uptake was reviewed by McMahon and Wilkens (1983) and nitrogen metabolism was reviewed by Claybrook (1983). The chemistry of growth and development has been covered by Yamaoka and Scheer (1970). Little information can be found which couples physiological and biochemical aspects during crustacean larval and postlarval growth and development.

This dissertation does not deal with the entire depth of crustacean metabolism. Therefore this literature review only examines topics which are relevant to this research. These topics include the effect of size and temperature on metabolic activity, an analysis of the O:N ratio, the effect of diet and starvation on biochemical composition and metabolic activity, and the biology of <u>Metapenaeus ensis</u>.

Nitrogen excretion results from the catabolism of proteins. The three major end products of nitrogen catabolism in animals are ammonia, urea, and uric acid (Campbell, 1973). Animals are classified on the basis of which compound is the dominant excretory product. Marine crustaceans are primarily ammonotelic, that is their primary nitrogenous waste product is ammonia (Claybrook, 1983). Ammonia, although toxic, is energetically the most efficient means of excreting nitrogen. It is primarily excreted through the gills as ammonium ion. Ammonia is highly soluble in water and easily diffuses into the surrounding medium (Horne, 1968; Jawed, 1969; Gerhardt, 1980). Urea, uric acid, and amino compounds may also be excreted (Regnault, 1979).

Values for the percentage of the various forms of nitrogenous waste within a species appears in Table II-1. The percentage of nitrogenous waste excreted as ammonia differs between species. The reported values range from 62 to 95%. Amino-nitrogen is usually the second most abundant form (Claybrook, 1983).

Most authors studying nitrogen excretion in Crustacea have measured only ammonia (Ikeda, 1977; Logan and Epifanio, 1978; Moffet and Fisher, 1978; Capuzzo and Lancaster, 1979; Nelson et al., 1979; Regnault, 1981; Blazka et al., 1982; Dall and Smith, 1986; and Sasaki et al., 1986). This research also quantified ammonia excretion as an indication of nitrogen excretion.

Metabolic activity may be affected by many diverse factors. Nitrogen excretion can be influenced by size (e.g. in *Macrobrachium lar*, Nelson and Kropp, 1985), temperature (e.g. in *Penaeus indicus*, Gerhardt, 1980), diet and starvation (e.g. in *Macrobrachium rosenbergii*, Clifford and Brick, 1978, 1979, 1983), stage of development (e.g. in _Homarus americanus_, Capuzzo and Lancaster, 1979), the stage of the molt cycle (e.g. in the sand shrimp, _Crangon crangon_, Regnault, 1979), and population density (e.g. in _Artemia salina_, Hernandorena and Kaushik, 1981).

Oxygen consumption also depends on many intrinsic and extrinsic factors. Intrinsic factors include the species, size (e.g. in _Macropetasma africanus_, Cockcroft and Wooldridge, 1985), recent feeding history (including quantity and quality of diet) (e.g. in _Macrobrachium rosenbergii_, Clifford and Brick, 1979), stage of larval development (e.g. in _Homarus americanus_, Capuzzo and Lancaster, 1979), the stage of the molt cycle (e.g. in _Homarus americanus_, Penkoff and Thurberg, 1982), and level of activity (e.g. in _Penaeus esculentus_, Dall, 1986). Extrinsic factors include salinity (e.g. in _Penaeus monodon_ and _Penaeus stylirostris_, Gaudy and Sloane, 1981), temperature (e.g. in _Palaemonetes vulgaris_, McFarland, 1965), lighting (e.g. in _Palaemonetes pacificus_, Emmerson, 1985), and oxygen tension (e.g. in _Penaeus japonicus_, Egusa, 1961).

Table II-1

Nitrogenous end products in crustaceans

	Waste product (%)				
Species	NH_3	aa-N	Urea	UA	Reference
Gammarus zaddachi	83.0	3.0	1.0	-	Dresel & Moyle, 1950
Gammarus pulex	70.0	3.0	9.0	-	"
Asellus aquaticus	62.0	10.0	-	5.0	"
Carcinides maenas	86.0	-	-	-	Needham, 1957
Neomysis rayii	82.1	11.1	1.4	-	Jawed, 1969
Palaemonetes varians	95.0	<5.0	-	-	Snow & Williams, 1971
Meganyctiphanes norvegica	*	15-30	-	-	Mayzaud, 1973
Acartia clausia	*	"	-	-	"
Macrobrachium rosenbergii	*	27-33.7	-	-	Clifford & Brick, 1979
Penaeus indicus	72.6	-	-	-	Gerhardt, 1980

* Ammonia reported as the dominant form

aa-N - Amino acid nitrogen

UA - Uric acid

1. The Effect of Size on Metabolic Activity

There is a quantitative relationship between body size and metabolic rate. As size increases the metabolic rate is also greater but the weight specific metabolic rate decreases (Bertalanffy, 1957). This relationship can be expressed by the allometric equation $M = aW^b$, where M is the metabolic rate (oxygen consumed or ammonia excreted) per unit time per individual, W is the weight of the animal and a & b are constants (a is a constant of proportionality and b is an exponential constant). When the logarithm of the weight specific respiration rate is plotted against the logarithm of the weight, the slope of the regression line (b - 1) is usually negative. This illustrates the decrease in weight specific metabolic rate with increasing size.

The allometric equation relating size to metabolic rate holds true for individuals within a species and generally within the same developmental stage. Differences in metabolism among species and between developmental stages may yield different constant values.

It was suggested that the constant b should approach 1 for respiration in small marine planktonic crustaceans, indicating a linear relationship between metabolic rate and weight, and be closer to 0.67, indicating proportionality between metabolic rate and surface area, in larger crustaceans (Zeuthen, 1953). Bertalanffy (1957) proposed that metabolic rates in decapods may be proportional to weight, to surface area (0.67 power of the weight), or intermediate between weight and surface area.

Most of the b values reported for oxygen consumption in crustaceans lie between the value for proportionality to surface area, 0.67, and the value for direct proportionality to weight, 1.00. A summary of these values appears in Table II-2.

The exponential constant, b, has also been calculated for rate of ammonia excretion. A summary appears in Table II-3.

From the wide variation in these results obviously the weight-metabolic rate relationship within a species may vary considerably. This may depend on factors such as the dietary condition (see section 4).

Blazka *et al*. (1982) suggested that in *Cyclops vicinus* and *Daphnia hyalina* the variability of excretion rate is greater than that of respiration. Nelson & Kropp (1985) also found a wide variation in weight specific excretion of *Macrobrachium lar*.

Table II-2

Oxygen consumption-weight relationship for crustaceans:

b values in the equation $M = aW^b$

Species	b	Reference
Homarus americanus	0.650	Logan & Epifanio, 1978
Macrobrachium rosenbergii	0.437 - 0.544	Clifford & Brick, 1979
Macrobrachium olfersii	0.620 - 0.690	McNamara _et al._, 1986
Macrobrachium heterochirus	0.660 - 0.710	"
Palaemon pacificus	0.780 - 0.867	Emmerson, 1985
Penaeus esculentus	0.848	Dall, 1986

M = rate of oxygen consumption/individual

W = weight of the individual

a & b = constants

Table II-3

Ammonia excretion-weight relationship for crustaceans:

b values in the equation $M = aW^b$

Species	b	Reference
Homarus americanus	0.650	Logan & Epifanio, 1978
Crangon franciscorum	0.131 - 0.376	Nelson *et al*., 1979
Macrobrachium rosenbergii	0.679 - 0.769	Nelson *et al*., 1977
Macrobrachium rosenbergii	0.407 - 0.743	Clifford & Brick, 1979
Macrobrachium lar	0.318 - 1.087 (mean = 0.8)	Nelson & Kropp, 1985
Cyclops vicinus	0.357 - 1.341	Blazka *et al*., 1982

M = rate of ammonia excretion/individual

W = weight of the individual

a & b = constants

2. The Effect of Temperature on Metabolic Activity

The effect of temperature on oxygen consumption

In most cases the rate of respiration increases with increasing temperature. This has been demonstrated in *Porcellio scaber* and *Porcellio pictus* (Wieser, 1972), *Penaeus indicus* (Gerhardt, 1980), and *Penaeus japonicus* (Kulkarni and Joshi, 1980). However, the response may vary depending on the temperature, species, and stage of development. Temperature may not affect the exponential constant, b, but simply displace the regression line relating rate to weight up or down (Dall, 1986).

McFarland and Pickens (1965) working on *Palaemonetes vulgaris* and Emmerson (1985) on *P. pacificus* both showed that there is no seasonal effect on the regression slopes (b value) for oxygen consumption. McFarland and Pickins reported no thermal acclimation, the rate of oxygen consumption being a function of test temperature and not thermal history. *Penaeus esculentus* also lacks short-term (one week) temperature acclimation (Dall, 1986). Lack of temperature compensation is unusual for aquatic poikilotherms.

In a temperature acclimation experiment with the entomostracan *Cyclops vicinus* the respiration rate for non-adapted individuals is similar to that for adapted individuals. It was concluded that metabolic acclimation to temperature takes less than one hour (Blazka *et al.*, 1982).

Blazka *et al*. (1982) found that oxygen consumption in marine zooplankton (*Cyclops vicinus* and *Daphnia hyalina*) is linearly related to body size at temperatures higher than 11 C, but proportional to 0.86 power of the size at 4 C, i.e., at lower temperatures an increase in size results in less of an increase in oxygen consumption. Cockcroft and Wooldridge (1985) (working on *Macropetasma africanus*), and Emmerson (1985) (working on *Palaemon pacificus*) found that the effect of temperature on the rate of respiration is independent of the size of the animal.

The Q_{10} value represents the factor by which metabolic rate will increase with an increase in temperature of 10 C. Emmerson found that the Q_{10} values for respiration in *Palaemon pacificus* increases with decreasing temperature. The same result was reported by Cockcroft and Wooldridge (1985) in *Macropetasma africanus*. According to Ganf and Blazka (1974) an increase in Q_{10} with decreasing temperature is a common response.

The effect of temperature on nitrogen excretion

The rate of nitrogen excretion increases with increasing temperature in many crustaceans, including the zooplankton species *Euphausia pacifica* and *Neomysis rayii* (Jawed, 1969), the terrestrial isopods *Porcellio scaber* and *Porcellio pictus* (Wieser, 1972), the cyclopoid *Thermocyclops hyalinus* (Ganf and Blazka, 1974), *Artemia salina* (Moffett and Fisher, 1978), and the shrimp *Penaeus* indicus (Gerhardt, 1980). In *Thermocyclops hyalinus* (Ganf and Blazka, 1974), and in *Cyclops vicinus* and *Daphnia hyalina* (Blazka *et al*., 1982), increasing temperature results in an exponential increase in ammonia excretion in these zooplankton. The same was found in *Artemia salina* by Moffett and Fisher (1978).

Temperature also alters the exponential constant, b, relating mass to excretion. With increasing temperature, between 16 and 28 C, *Penaeus indicus* experiences an increase in nitrogen excretion; this increase is more pronounced with increasing shrimp size (Gerhardt, 1980).

Johannes and Webb (1965), Webb and Johannes (1967), and Jawed (1969) have reported that the proportion of amino-nitrogen excreted increases with increasing temperature.

According to Ganf and Blazka (1974), with an increase in temperature, the rate of increase in ammonia excretion may lag behind the increase in oxygen consumption. They suggested that this may reflect a lag between protein catabolism and ammonia excretion.

3. Analysis of The O:N Ratio in Marine Organisms

Harris (1959) was the first to determine O:N ratios for marine organisms, calculating a value of 7.7 for mixed zooplankton. Since then the O:N ratio has been frequently applied to Crustacea as well as other taxonomic groups. For example, Barber and Blake (1985) showed how the O:N ratio could be applied to determine the changes in substrate catabolism during the reproductive cycle in the bay scallop, *Argopecten irradians*, linking the O:N ratio to changes in biochemical composition. Kremer *et al.* (1986) used the O:N ratio as an indicator of protein catabolism in five species of ctenophores.

The atomic ratio of oxygen consumed to nitrogen excreted (the O:N ratio) by marine organisms is more easily and accurately determined than the respiratory quotient (ratio of carbon dioxide produced to oxygen consumed) (Dall and Smith, 1986; Snow and Williams, 1971). It is because of the difficulty in measuring dissolved carbon dioxide. Both ratios provide an indication of metabolic substrate utilization.

A high O:N ratio is an indication of carbohydrate and lipid catabolism. If only carbohydrates are being oxidized, the ratio approaches infinity. The greater the proportion of proteins utilized in catabolism, the lower the O:N ratio will be. Conover and Corner (1968) calculated a theoretical minimum O:N ratio (resulting from the catabolism of pure proteins) of 8.0. Clifford and Brick (1978) stated that lower values are theoretically impossible. However, the value of 8.0 is calculated from the catabolism of mammalian proteins. According to Mayzaud (1973) marine plankton have a greater proportion of basic amino acids, so that the C:N ratio in the body protein is lower, allowing an O:N ratio as low as 4.0.

In a time course experiment, nitrogen excretion may decrease with increasing duration of the experiment, although the total amount of ammonia excretion remains constant (Borgne, 1979). The oxygen:ammonia ratio is independent of the length of experiment. It was therefore concluded that the use of this ratio as an index of substrate catabolism is justified.

Conover and Corner (1968) investigated seasonal variation in biochemical composition and O:N ratio in selected copepods. They discovered a close link between the seasonal differences in O:N ratios and the life cycle of the animal. Also the ratio is linked to its biochemical composition, especially fat content. During phytoplankton blooms herbivorous copepods store fat and have an O:N ratio of 10 to 30. After the bloom the animals utilize stored fat as the energy source and an O:N ratio of greater than 150 was observed. As fat reserves are used protein is more heavily utilized, and the O:N ratio declines to less than 20. However, in omnivorous copepods, respiration and excretion are both higher, but the O:N ratio rarely rises above 20. In this study, the weight specific rate of nitrogen excretion increases more rapidly than the rate of respiration when body weight decreases. A brief review of O:N ratios in other copepods is given by Corner and Cowey (1968). A more recent study on a zooplankton community (Ganf and Blazka, 1974) gives a value of 17.3, which remains constant during the study period. Marine pelagic organisms possibly favor lipid storage and metabolism because of its buoyancy and higher caloric yield. This should be kept in mind when comparing organisms from other habitats (Clifford and Brick, 1983).

An increase in O:N ratio with starvation in carnivores suggests that there is an increase in carbohydrate or lipid metabolism. Well fed animals adjusted to a protein rich diet may consume enough protein for production and catabolism. In carnivores such as *Cyclops vicinus*, excretion rate increases with increasing food concentration. In herbivores such as *Daphnia hyalina*, nitrogen excretion decreases when food is more abundant, so that the O:N ratio increases (Blazka *et al.*, 1982). This is supported by Ikeda (1977) who found that the O:N ratio in herbivores decreases with starvation.

Mayzaud (1973) suggested that changes in O:N ratio can be accounted for more by a change in the excretion rate, rather than by a change in the respiration rate. This is supported by Wieser (1972) who found that during 4 days of starvation in terrestrial isopods the oxygen consumption remains constant while in most cases the ammonia excretion increases. However this case is the exception rather than the rule. There is usually a decrease in respiration during starvation (e.g. Bayne and Scullard, 1977; Ikeda, 1977; Blazka _et al_., 1982; Clifford and Brick, 1983; Nelson and Kropp, 1985; Anger, 1986)

The O:N ratio may be linked to temperature but differences due to temperature tend to be species specific. Wieser (1972), comparing terestial isopods, found that at 20 C the O:N ratio of _Porcellio pictus_ is always higher than that at 30 C, whereas the opposite is true for _Oniscus asellus_. Conover and Corner (1968) suggested that increasing temperature results in a higher O:N ratio in animals with a high lipid content but a lower O:N ratio in lean animals. This is supported by Weiser's (1972) data on _Porcellio pictus_, a lean animal with few lipid or carbohydrate reserves, and _Oniscus asellus_, an animal with greater reserves. The O:N ratio found in the study ranges from 9 to 150.

Snow and Williams (1971) found O:N ratios of 6.1 in winter and 34.2 in summer for the prawn _Palaemonetes varians_. This indicates a shift from protein catabolism to utilization of carbohydrate or fat as an energy source.

The O:N ratio may be used to determine the most efficient dietary protein level in experimental diets, as illustrated by Clifford and Brick (1978, 1979). The shrimp _Macrobrachium rosenbergii_ has O:N ratios ranging from 17.8 to 33.7 (Clifford and Brick, 1978). These figures are inversely correlated to dietary protein levels and fat to carbohydrate ratio. Such an inverse correlation was confirmed in their subsequent study when O:N ratios vary between 21.9 and 46.8 (Clifford and Brick, 1979).

Although the O:N ratio is a useful indicator of substrate catabolism, it is not without limitations. Clifford and Brick (1983) pointed out that the O:N ratio provides only vague and qualitative information on substrate catabolism. They also noted that although a particular component may be the primary or preferred catabolite, usually a mixture of substrates is oxidized. Changes in level of activity will also drastically alter the O:N ratio by changing the rate of oxygen consumption, while the substrate catabolism may not be affected.

4. The Effect of Diet and Starvation on Biochemical Composition and Metabolic Activity

The physiological and biochemical effects of diet and starvation may vary from species to species, between larval stages, and during the period of starvation (Anger *et al.*, 1985). It may also depend on environmental factors and dietary condition prior to starvation (Aldrich, 1975; Clifford and Brick, 1983; Regnault, 1981).

Basically there are two types of starvation experiments found in the literature. One design involves an initial starvation for a period of time generally not exceeding 3 days, followed by feeding. This design is often used to examine the short term effect of starvation and to evaluate experimental diets by examining their physiological effects on starved animals. In the second design animals are starved for an extended period of time and physiological parameters are measured at routine intervals throughout the experimental period. This type of experiment is specifically concerned with the long term effect of starvation.

Borgne (1979) made the distinction between short term starvation and long term starvation. Animals frequently experience short term starvation in the natural environment, the result of which is a small decrease in metabolic activity, involving both respiration and nitrogen excretion rates (Ikeda, 1977). The length of time before long term starvation comes into effect may depend on size, species, and initial dietary condition. Mayzaud (1973) suggested three metabolic levels of resistance to starvation in selected zooplankton: an initial lipid-protein based metabolism; an intermediate level represented by a decreased O:N ratio, indicating greater protein catabolism, but without great physiological stress; and a final phase characterized by physiological disequilibrium. Two types of metabolic response to starvation were suggested. Species with a low biomass turnover rate that utilize seasonal food reserves have a greater resistance to starvation (e.g. *Meganyctiphanes norvegica*), than species with a high turnover rate and no dietary reserves (e.g. *Acartia clausi*).

The reduction in metabolic activity associated with starvation is more pronounced in smaller animals. Smaller animals have proportionally fewer reserves, so that a greater reduction in catabolism will enable them to delay utilization of stored nutrients (Clifford and Brick, 1983).

The effect of diet and starvation on oxygen consumption

In the absence of feeding the respiration rate of crustaceans decreases (Ikeda, 1977; Aldrich, 1975). The respiratory response of crustaceans to long term starvation is species specific (Mayzaud, 1973) and usually follows one of two patterns. That of a high initial decrease followed by a leveling off (e.g., *Penaeus esculentus*, Dall and

Smith, 1986; *Hyas araneus*, Anger, 1986), or that of a small initial decrease followed by a rapid decrease after a longer period of starvation (e.g., *Crangon crangon*, Regnault, 1981).

In the first pattern, the time taken to level off and the rate at which respiration levels off may vary. The oxygen consumption rate of *Penaeus esculentus* (Dall and Smith, 1986) decreases to 70% of the initial level within 5 days and thereafter remains constant for 15 days of starvation. The respiration rate of *Penaeus japonicus*, after a slight insignificant increase, decreases continually over a 10 day period to about 60% the initial level, and remains constant till the end of an experimental period of 15 days (Kulkarni and Joshi, 1980). In a prolonged starvation experiment, the respiration rate of *Pandalus platyceros* decreases by 50% in 40 days (Whyte *et al.*, 1986). The respiratory rate of starved *Hyas araneus* decreases rapidly at the onset of starvation, reducing by 83 to 88% after 20 days (Anger, 1986). During an 80-hour starvation period in *Cyclops vicinus* oxygen consumption falls by 45%. Most of the decrease took place within the first 30 hours (Blazka *et al.*, 1982).

In experiments with zooplankton, Mayzaud (1973) found that the oxygen consumption rate of *Acartia clausi* experiences a sharp decrease within the first 6 hours, following the first pattern, while the response of *Meganyctiphanes norvegica* is similar to the second pattern, remaining constant for 25 hours before decreasing.

Crangon crangon also follows the second pattern of response to starvation, i.e., a small initial decrease followed by a rapid decrease after a lag period (Regnault, 1981). During a 30 day starvation experiment, the oxygen consumption decreases about 10% in the first 5 days, then drastically to 43% of the original level by day 14, and remains at this level for the rest of the experimental period.

The percentage increase in respiration with feeding after a period of starvation depends on the dietary condition before starvation (Aldrich, 1975), duration of the starvation period (Anger *et al.*, 1985), and the diet fed (Nelson *et al.*, 1985). The increased rate of oxygen consumption associated with feeding is known as the specific dynamic effect (Kleiber, 1961).

Fed *Crangon franciscorum* have a metabolic rate higher than those starved for 48 hours (Nelson *et al.*, 1985). The oxygen consumption of fed shrimp is 14 to 43% higher, depending on the type of diet. For *Macrobrachium rosenbergii* the increase has been reported to range from 7 to 39% (Nelson *et al.*, 1977) and from 15 to 25% (Clifford and Brick, 1978).

The decrease in the rate of respiration with starvation may be restricted to active metabolism, but have little or no effect on routine metabolism (Barclay *et al.*, 1983). Dall and Smith (1986) found that over a 15 day starvation period in *Penaeus esculentus*, the oxygen consumption rate during the day decreases very little , but the rate during the night decreases significantly. Shrimp are nocturnal animals. The decrease in respiration may also be more apparent in earlier more active life stages, rather in later, more sedate, and benthic stages, as shown in <u>Homarus americanus</u> by Capuzzo and Lancaster (1979). Wieser (1972) (working on <u>Porcellio scaber</u>, <u>P. pictus</u>, and <u>Oniscus asellus</u>) found oxygen consumption to remain constant during a four-day starvation period. It may be because the respiration rate was measured during the inactive daytime period alone.

<u>The effect of starvation on nitrogen excretion</u>

The pattern of change in ammonia excretion with starvation is highly dependent on the species studied (Wieser, 1972). The short term effect of starvation is generally a reduction in ammonia excretion (Nelson <u>et al.</u>, 1979; Regnault, 1981; Clifford and Brick, 1983). With prolonged starvation ammonia excretion tends to increase. Ammonia excretion of <u>Crangon crangon</u> decreases within 24 hours of starvation to 75% of the initial value. It remains at this level for the first 5 days and then increases to 30% above the initial level by the eleventh day, a level which is maintained for 30 days of starvation (Regnault, 1981). It was suggested that an excretion rate 75% of the initial value represents the basal level of excretion, whereas after 5 days the effect of starvation comes into play.

After 4 days of starvation the nitrogen excretion of *Macrobrachium rosenbergii* is lower than subsequently fed groups. But after 8 days of starvation the nitrogen excretion increases 58% (Clifford and Brick, 1979, 1983). In *Meganyctiphanes norvegica* and *Acartia clausia*, an initial decrease in excretion with starvation is also followed by an increase (Mayzaud, 1973).

However, Dall and Smith (1986) noted no initial decrease in nitrogen excretion in a 15 day starvation experiment with *Penaeus esculentus*. This may be because their first measurements were made after 5 days of starvation. The nitrogen excretion increases by 46-73%.

The rate of ammonia excretion is related to feeding level. Blazka *et al*. (1982) observed the lowest rate of ammonia excretion in mixed pond zooplankton (species of Cyclopoida, Cladocera, and Rotatoria) at low natural feeding levels.

The effect of starvation on ammonia excretion is usually more marked at a higher temperature (Wieser, 1972). This is manifested by an increase in ammonia excretion, but may be followed by a decrease in excretion.

In *Cyclops vicinus*, there is an increase in rate of ammonia excretion with fasting at temperatures higher than 10 C but a decrease was observed at 4 C (Blazka *et al*., 1982). This is possibly associated with utilization of lipids during the winter.

The effect of diet on nitrogen excretion

Nitrogen excretion may alter drastically between starved and fed individuals. However, within the fed state, the feeding habits and quality of diet may also affect nitrogen excretion.

Blazka _et al._ (1982) found that in mixed pond zooplankton the lowest rate of ammonia excretion was at low natural feeding levels. In animals which have been feeding excessively the ammonia excretion increases. In well fed animals protein may not have to be conserved, so that the amount consumed may be sufficient for both growth and catabolism. Blazka suggested that this relationship may only apply to carnivores.

The rate of ammonia production in _Macrobrachium rosenbergii_ was found to be unaffected by diet quality, but only related to the weight of the individual (Nelson _et al._, 1977). This finding is in direct contrast to that of Clifford and Brick (1979). They found that there is a tremendous variation in the excretion rate of animals fed with different experimental diets. These differences are related to % protein in the diet and to the lipid: carbohydrate ratio. This was supported by Nelson _et al._ (1979) who found that the ammonia excretion rate in _Crangon franciscorum_ is influenced by both the quality of the diet and the amount consumed. The ammonia excretion rate in _Macrobrachium lar_ is also influenced by type of diet, although the basic weight-metabolic rate relationship is not affected (Nelson and Kropp, 1985). Takahashi and Ikeda (1975) found that the rate of ammonia excretion in Euphausia pacifica and Metridia pacifica is proportional to the amount of food ingested.

Moffett and Fisher (1978) found no significant difference in ammonia excretion between fed and unfed Artemia salina.

Effect of starvation on substrate utilization

Changes in oxygen consumption and ammonia excretion during starvation are related to the use of body reserves for survival and are therefore accompanied by changes in body composition (Clifford and Brick, 1983; Regnault, 1981). A description of fasting substrate metabolism provides information on the hierachy of substrate utilization (Clifford and Brick, 1983).

After 30 days of starvation, the total protein content of Crangon crangon decreases by 50% (Regnault, 1981). The initial O:N ratio is 27. During the first 5 days of starvation the O:N ratio increases to 30-65, suggesting the use of carbohydrate and lipid reserves. It then decreases to 8, pointing to a reliance on protein catabolism and accounting for the decrease in protein content.

A similar pattern is seen with Macrobrachium rosenbergii (Clifford and Brick, 1978, 1979, 1983; Nelson et al., 1977). Little protein is utilized during the first 4 days of fasting. Rather primarily carbohydrates are utilized and an O:N ratio of 100 results. After 8 days of starvation the O:N ratio is 61, indicating a continued, although lesser, reliance on carbohydrates.

The O:N ratio of Porcellio pictus decreases with starvation indicating catabolism of constituent proteins and probably a lack of lipid or carbohydrate reserves (Wieser, 1972). Oniscus asellus has less of a dramatic decrease in O:N ratio with starvation. It is probably utilizing reserve lipids or carbohydrates.

Pandalus platyceros can withstand long periods of starvation, up to 84 days, by relying on lipid reserves (Whyte, 1986). During this period lipids provide 73% of the required calories, protein 21%, and carbohydrates 6%.

Although many crustaceans may rely on one particular biochemical component during starvation, most likely a mixture of substrates is catabolized (Clifford and Brick, 1983). Penaeus esculentus resorts readily to protein catabolism (Dall and Smith, 1986). After 5 days of starvation the O:N ratio falls from 14 to 7 and remains at this level for 15 days. In Hyas araneus, protein and lipid levels decrease by 38 to 58% and 35 to 40% respectively. The absolute amount of metabolized protein is three times greater than lipid (Anger, 1986).

The body water content of starved Crangon crangon increases during starvation from 72 to 81% (Regnault, 1981). Pandalus platyceros also takes up moisture during starvation (Whyte *et al.*, 1986). The opposite effect was seen in *Hyas araneus*. The percentage dry weight of larvae exposed to continued starvation is greater in each successive larval stage (Anger, 1986).

5. Metabolic Activity and Biochemical Composition in Crustacean Larvae

Very few studies on crustacean metabolism include the larval stages. The larvae are usually more difficult to obtain and to work with, because of their small size and usually more specific dietary requirements. Larval rearing of the penaeid shrimp has only been possible on a large scale since the early 1960's (Shigeno, 1978). The transitional nature of the larval stages in the life cycle, and associated physiological changes make this aspect of crustacean biology fascinating.

The rate of oxygen consumption may vary between the larval stages. This variation may not simply be accounted for by an increase in size, as the larval stages may have different life styles, which may also differ from that of the juveniles or adults.

Reeve (1969) examined the growth and energy conversion in larvae of the prawn, Palaemon serratus. Physiological and biochemical parameters were not quantified.

Logan and Epifanio (1978) studied the energetics of larval and juvenile Homarus americanus. They found that the weight specific metabolism is highest in the early larval stages. This study is in contrast to Capuzzo and Lancaster (1979)(see below). Capuzzo and Lancaster (1979) suggested this difference may be due to the insufficient sensitivity of the Gilson respirometer used by Logan and

Epifano to measure respiration in all larval stages. Capuzzo and Lancaster used a micro-respirometer for the early larval stages. The specific dynamic action, reported by Logan and Epifanio, is 148% for both larvae and juveniles.

Capuzzo and Lancaster (1979) quantified biochemical composition, respiration rate, and ammonia excretion rate, and identified possible changes in energy utilization in the larval and first postlarval stages of the American lobster, Homarus americanus. The percent dry weight increases throughout the larval stages. They found that with progressive larval stages the rate of change in metabolic rates is greater than the rate of increase in larval size, i.e. an increasing weight specific rate of oxygen consumption in successive larval stages (b = 1.24), indicating an increasing energy demand. The O:N ratio decreases in the later larval stages, decreasing from 26.7 to a minimum of 22.1, accompanied by a decrease in the percentage lipid content to suggest a greater dependence on protein catabolism. Weight specific respiration and ammonia excretion rates are higher in animals immediately after feeding than in those starved for 24 hours. The rate of oxygen consumption in fed animals is 23 to 64% greater than that of starved animals. Sasaki et al. (1986) confirmed these results and also examined embryogenesis and morphological aspects in greater detail. They suggested a reliance of newly settled juveniles on lipid reserves during their acclimation to a benthic environment.

Anger *et al.* (1985) found that stage I larvae of *Homarus americanus* can withstand a 1-2 day period of starvation but are never able to recover upon feeding after 5 days of starvation. They suggested that the ability to recover is related to the capacity to restore lipid reserves depleted during starvation.

Stephenson and Knight (1980) found b values for the oxygen consumption of larval, postlarval, and juvenile *Macrobrachium rosenbergii* to be 0.904, 0.984, and 0.386 respectively, i.e., juveniles show a much greater decrease in respiration rate with increasing size.

Anger (1984) examined the changes in particulate organic and inorganic matter in relation to growth and molting in larval and juvenile *Hyas araneus*. Larval and early postlarval growth was described as an exponential function of the stage of development. The ash and percent dry weight values are higher in juveniles than in larvae.

Anger and Jacobi (1985) and Jacobi and Anger (1985b) quantified respiration rate, dry weight, and elemental composition in the larval stages of two species of spider crabs, *Hyas araneus* and *Hyas coarctatus*. They suggested that during the zoeal stages lipids are accumulated to a greater degree than proteins. The composition of the megalops stages remains constant. The respiration response to extrinsic factors is different for each larval stage. A similar conclusion was made by McNamara *et al.* (1986) for zoeal, postlarval, and adult *Macrobrachium olfersii*.

Larval stages of *Hyas araneus* and the megalopa of *H. coarctatus* have Q_{10} values increasing with decreasing temperature. But the Q_{10} values for the zoeal stage of *H. coarctatus* remains constant or decreases with decreasing temperature (Jacobi and Anger, 1985a).

There is no decrease in the respiration rate with starvation in larval *Hyas araneus* (Anger, 1986). Respiration rate in starved animals increases with each successive molt stage.

During starvation, the zoea larvae of *Carcinus maenas* preferentially utilize lipids as a source of energy. Body protein is only utilized when no lipids are available. Carbohydrates do not make an important contribution during starvation (Dawirs, 1987).

6. The Biology of *Metapenaeus ensis*

The species *Metapenaeus ensis* (de Haan) is commonly known in Hong Kong as the sand shrimp. *Metapenaeus ensis* is sometimes confused with *Metapenaeus monoceros*. It is possible that the species known as *M. monoceros* in Taiwan is actually *Metapenaeus ensis* (Hall, 1958). This has also been suggested by Yu and Chan (1986). The following review will only deal with research published under the name of *M. ensis*. Very little published research is available.

Metapenaeus ensis is found from the Bay of Bengal to Japan and south to Australia (Indo-West Pacific south of Malacca Strait, Hall, 1958). It is essentially restricted to the continental shelf, ranging inland into estuaries, a depth of 8-64 m (Yu and Chan, 1986). *Metapenaeus ensis* is a commercially important species both to the fisheries and mariculture industries in Hong Kong (Cheung, 1964). The species *M. ensis* owes its popularity in culture to a relatively long spawning season (approximately from the end of April to the end of October, Cheung, 1964), tolerance to low temperature, ability to survive for a long period out of water (an important factor where market acceptance depends on live products), and the availability of gravid females in the local market. *Metapenaeus ensis* is also known to be cultured in Singapore, the Phillipines, Taiwan, Malaysia, India, Pakistan, New Caledonia, and China. The Indo-West Pacific shrimps are one of the most lucrative fishery resources in the region (Racek, 1972) and the South China Sea is listed as one of four of the world's major shrimp fishery areas (Wickins, 1976).

The fisheries biology of adult *Metapenaeus ensis*, caught in waters surrounding Hong Kong, was studied by Cheung (1964). He examined population recruitment, size, and ovarian development. The distribution of *M. ensis* in waters surrounding Hong Kong was found to be strongly influenced by water of a lower salinity from the Pearl River estuary. The juveniles prefer the lower salinity and the adults prefer the oceanic water. The greatest abundance of shrimp with maturing or mature ovaries was found in the spring and autumn (approximately March and August). Analysis of carapace length-frequency curves suggested a life span of 15 to 20 months.

Ong (1969) gave the first account of larval life history, detailing the rearing procedure and the larval morphology to the mysis III stage. However, an incomplete larval life history was reported.

Tseng *et al*. (1979) explained the complete larval life history and the rearing conditions of this shrimp at the Marine Science Laboratory of the Chinese University of Hong Kong.

During larval development *Metapenaeus ensis*, like other penaeid shrimps, experiences a complicated series of morphological and behavioral changes. *Metapenaeus ensis* larvae undergo a number of molt stages before becoming postlarvae. These molt stages can be divided into three main developmental stages on the basis of morphology and feeding behavior. *Metapenaeus ensis* has 6 naupliar (N I-VI), 3 protozoeal (PZ I-III), and 3 mysis (M I-III) larval stages between the egg and the first postlarvae (Ong, 1969; Tseng *et al.*, 1979). The planktonic nauplius is non-feeding, living on egg yolk reserves. It is common hatchery practise to feed the protozoeal stages with unicellular algae and begin feeding zooplankton during the late mysis stage. During the postlarval stage the planktonic larva settles to the bottom becoming a benthic omnivore. This takes place at about postlarva 5 (5-day old postlarva).

Chan (1984) examined the effects of salinity, temperature, diet, and a number of chemicals on the development of *Metapenaeus ensis* during culture. The temperature range for hatching is 14-36 C. The optimum salinity for spawning and hatching is 30 ppt. The optimum salinity for juvenile growth (31 mm length) is 30-40 ppt.

Chan also examined the combined effect of salinity and temperature on respiration of juveniles. To determine oxygen consumption, Chan used blackened dissolved oxygen bottles with a single dissolved oxygen meter reading after a 12 hour test period. A salinity range of 5-35 ppt does not affect rate of respiration. The rate of oxygen consumption increases over a temperature range of 12-36 C, ranging from 1.12 mg O_2 /g dry weight/hour to 4.99 mg O_2 /g dry weight/hour, at 30 ppt salinity. The Q_{10} values decrease as the temperature increases. Between 12-22 C the

Q_{10} value is 1.92-2.58. Between 26-36 C the Q_{10} value is 1.57-1.79. In the equation relating metabolic rate to weight, $M=aW^b$, the constant b is 0.562.

Chu and Shing (1986) investigated ingestion of *Artemia* nauplii by the mysis and postlarvae of *Metapenaeus ensis*. Their data show very little *Artemia* consumption at the mysis III stage and suggest a shift to raptorial feeding behavior between the postlarva 1 and postlarva 4 stages.

Chu and So (1987) further investigated salinity tolerance during larval development. The optimum salinity for hatching was confirmed to be 30 ppt. Tolerance to low salinity increases in each successive larval stage.

Chapter III

Ontogenetic Changes in Metabolic Activity and Biochemical Composition in *Metapenaeus ensis*

Introduction

As a result of high market demand the cost of shrimp has escalated. To exploit the opportunity for vast financial gain, research efforts have increasingly focused on shrimp culture. Grow-out operations have been more successful than hatchery operations. Traditionally grow-out ponds were stocked with wild caught postlarvae or postlarvae produced from wild caught gravid females (Lawrence *et al.*, 1985). Fear of a resulting decline in shrimp fisheries has resulted in restrictions on removal of postlarvae and gravid females from their natural habitat. Therefore aquaculture operations are forced to develop hatchery technology. Successful rearing of larvae depends on an understanding of their physiology.

The larval development of penaeid shrimps is amongst the most complex found in crustaceans (Wickens, 1976). In 1942, Hudinaga made the original breakthrough in larval culture in *Penaeus japonicus* (Shigeno, 1969). This knowledge of shrimp culture has been considerably expanded in the past twenty years (see Wickens, 1976, 1986 for reviews). Nevertheless very few studies dealt with metabolic activity of the larvae. The larvae of penaeid shrimp experience a complicated sequence of behavioral changes during development. Changes in physiological parameters and biochemical composition might also be expected.

This research was undertaken to examine oxygen consumption, ammonia excretion, and the biochemical composition in selected ontogenetic stages of the shrimp <u>Metapenaeus ensis</u>. The metabolic response to a 24 hour starvation period and upon feeding was studied. O:N ratios were calculated. These data should indicate any changes in substrate utilization during larval and postlarval development.

Materials and Methods

Animals

Gravid female Metapenaeus ensis were obtained from local fish markets and transported to the Marine Science Laboratory. The females were individually placed in 500 l aerated fiberglass tanks at a salinity of 30 ppt. The water temperature in the outdoor tanks during the experimental period (June to October, 1986) was 26-34 C. The shrimp usually spawned in the early morning of the day following purchase and the spawners were then removed from the tanks. In this way each batch of larvae studied came from a single spawn.

The eggs hatched by about noon of the same day. Unicellular algae were introduced during the non-feeding naupliar stage so that the shrimp could begin feeding immediately after molting to the first feeding stage, protozoea I. A mixture of unicellular algae was used, including a blue-green alga, Arthrospira sp. Live algal cultures were supplemented with a commercial diet, BP diet (Nippai Brand). It was found that Metapenaeus ensis can rely on unicellular algae throughout the larval development (Chu and Shing, 1986). Freshly hatched nauplii of the brine shrimp, Artemia salina, were introduced to the tank during the mysis III stage so that they would be available when the larvae became the raptorial postlarvae. The postlarvae were reared on brine shrimp naulpii throughout the remainder of the experimental period, i.e. until when they were 9 day old postlarvae.

Selected ontogenetic stages were chosen to be studied. Metapenaeus ensis has 6 naupliar, 3 protozoeal, and 3 mysis stages before becoming a postlarvae. According to Chan (1984), the eggs hatch in 12-14 hours at 27-33 C. The larvae spend about 14 hours in the naupliar stages, 3-4 days in the protozoeal stages, and 4-5 days in the mysis stages. The egg was studied as well as a representative molt stage for each of the three larval stages and two postlarval stages, to make a total of 6 stages studied. The eggs were studied 3-8 hours after spawning. Examination under the microscope showed that they were at the morular or pre-nauplius stage of embryonic development. It was observed that within each larval stage the larvae tend to spend the longest period of time in the final molt stage. Thus, these stages were chosen for experimentation. It was impossible to choose only one naupliar stage for experimentation because of the short period of time available during the nauplius stage and therefore the unlikelihood that the nauplii would remain within one molt stage for the duration of the experiment. The postlarvae switch from a planktonic to a benthic existence at about postlarva 4-5. A postlarval stage before and after this shift in life style was chosen. The stages chosen were the egg (E), the nauplius 5 and 6 (N V/VI), the protozoea 3 (PZ III), the mysis 3 (M III), 3-day old postlarva (PL 3), and 9-day old postlarva (PL 9).

The parameters to be determined were body length and wet weight, biochemical composition, oxygen consumption, ammonia excretion, and, for those stages which fed, the response of metabolic activity to a 24-hour starvation period.

Measurement of body length and individual wet weight

To determine mean wet weight, a sample of larvae were throughly washed, to remove algae and *Artemia*, and filtered through a millipore filter, weighed, and counted. The sample size for each batch was ~500 for the egg, ~220 for the nauplius, ~200 for the protozoea, ~110 for the mysis, and ~75 for postlarvae 3 and 9. Mean length was measured with a micrometer under a microscope. Thirty larvae from a single spawn were measured to obtain a mean. Determinations were made on six batches of larvae.

Analysis of biochemical composition

Samples of the selected ontogenetic stages were taken from the same spawns used for determinations of physiological parameters. They were washed to remove excess algae and *Artemia*. Excess water was removed by passing samples through a millipore filter with a suction pump. Wet weight was taken and then a portion of the sample was reserved for determination of water content and ash weight. The remainder of the samples was frozen, freeze dried, and stored in sealed vials at 4 C until biochemical analysis.

1. Water content

About 100 mg wet weight of the sample was dried, in a weighed crucible, in the oven at 60 C for 24 h. The sample was weighed after cooling in a desiccator.

2. Ash weight

The same sample used for determining dry weight was then ashed in the furnace at 500 C for 24 h. The sample was weighed after cooling in a desiccator (Raymont et al., 1964). Ash free dry weight is the difference between dry weight and ash weight.

3. Protein

Protein level was determined using the biuret reagent according to Gornall et al. (1949). The biuret method involves a colorimetric reaction with copper sulfate. Bovine serum albumin was used as the standard. A sample size of approximately 4 mg freeze dried weight was required for analysis.

4. Lipid

The lipid fraction of the freeze dried sample was extracted with a chloroform-methanol mixture according to the procedure described by Sasaki and Capuzzo (1984). Lipid level was then determined using the sulphophosphovanillin method according to Barnes and

Blackstock (1973). This is a colorimetric method relying on the reaction of lipids with sulfuric acid, phosphoric acid, and vanillin to form a red complex. Cholesterol was used as the standard. A sample size of 5 mg freeze dried weight was required.

5. Carbohydrate

Carbohydrate was determined using the colorimetric phenol-sulfuric acid method (Dubois *et al.*, 1956 and Raymont *et al.*, 1964). A glucose standard was used. A sample size of 1 mg freeze dried weight was required.

Determination of oxygen consumption and ammonia excretion

For determination of metabolic activity a sufficiently large larval sample was removed from the culture tank. The number depended on the stage being analyzed and decreased with increasing larval size. About 1000 eggs, 50-55 N V/VI, 30-35 PZ III, 15 M III, 10 PL 3, and 7 PL 9 were used. The decline in the number of individuals required was due to increasing oxygen consumption with each larval stage. The larvae were observed under a dissecting microscope to ensure they were all in the selected larval stage. They were removed from the culture tank and transferred to beakers containing artificial sea water of 30 ppt salinity and 25 C. The water was removed and replaced several times to ensure the removal of all algal cells and *Artemia*. The seawater was made with hw-Marinemix

+ Bioelements, Wimex brand. The artificial seawater was prepared fresh daily and passed through a 20 µm mesh filter before use.

The eggs and the nauplius V/VI are non-feeding stages and their oxygen consumption and ammonia excretion were only measured once. The eggs hatched on the same day that they were spawned, and remained in the nauplius stage for about 14 h. No acclimation was performed for these stages before determination of physiological parameters.

Metabolic activity was determined twice for the PZ III to PL 9 stages. The shrimp were removed from the culture tank, as explained above, and maintained in a water bath at 25 C without food for 24 h. The oxygen consumption and ammonia excretion rates were measured. The larvae and postlarvae were then fed for 2 hours and oxygen consumption and ammonia excretion were measured again. Care was taken to remove all trace of food and place the animals in fresh seawater before determination was repeated.

The PZ III and the M III stages were fed with algal culture of the diatom <u>Chaetoceros gracilis</u>. The PL 3 and 9 were fed with freshly hatched nauplii of <u>Artemia salina</u>. The feeding levels were approximately 30,000 cells of <u>C. gracilis</u> per ml or ten <u>Artemia</u> nauplii per ml. The volume of water used was about 400 ml.

The experimental apparatus for oxygen consumption measurement is shown in Figure III-1. A microcathode oxygen electrode, Radiometer model number E 5046, and oxygen meter, Strathkelvin Instruments model number 781b, were used for oxygen consumption measurement. The shrimp to be studied were placed in a glass vial, acting as the respiration cell. The oxygen electrode was inserted into the glass vial. The volume of the glass vial, was approximately 7 ml. The exact volume was measured after each experiment. With the insertion of the electrode into the vial it became a closed chamber. It was necessary to insert a needle through the rubber stopper holding the electrode in order to relieve pressure in the vial. After the pressure was stabilized the needle was removed to seal the vial. A film of petroleum jelly was smeared over all the seams to ensure against any leakage. The vial was placed in a constant temperature water bath maintained at a temperature of 25 C. A magnetic stirring bar was placed in the vial, and the vial in the water bath was suspended over a magnetic stirrer to keep the water well mixed. On top of the stirring rod the vial was fitted with a small perforated platform and the stirring was maintained at a very low, gentle level to ensure that larvae were not damaged.

The oxygen meter was calibrated before the experiment by using oxygen saturated seawater, with a value of 5.06 ml oxygen/l as the saturation concentration of seawater at 30 ppt and 25 C (Sverdrup et al., 1970). A sodium borate solution with a pinch of sodium sulfite was used to set the meter to a zero reading. Artificial seawater which had been aerated till saturated with oxygen (about 30 minutes) was used for the experiments. During the experiment the oxygen concentration was noted every 5 minutes. The decrease in oxygen concentration stabilized after 20-30 minutes. Each experiment lasted two to two and a half hours. Within this period the oxygen concentration did not drop below 70% saturation as oxygen consumption may be affected by oxygen tension (Bridges and Brand, 1980; Dall, 1986). This technique for measurement of oxygen consumption is similar to that described by Davenport (1976).

The experiment was always performed at roughly the same time of day to avoid any possible diurnal variation in oxygen consumption (Kader, 1962; Dall, 1986; Dall and Smith, 1986). The egg and the nauplius V/VI were studied between 6 am and 12 noon, depending on the time of the spawn. The other stages were studied between 10 am and 6 pm. Consistency in the time of experiment may also be important for measurement of ammonia excretion (Ganf and Blazka, 1974; Dall and Smith, 1986).

The specific dynamic action was calculated using the following equation (Kleiber, 1961):

$$\frac{K_1 - K_2}{K_2} \times 100 = \%\ \mathrm{SDA}$$

K_1 = oxygen consumption after feeding

K_2 = oxygen consumption after 24 h starvation

Ammonia excretion was measured concurrently with oxygen consumption. After the oxygen consumption measurement the animals were removed from the respiration cell and a 5-ml water sample taken for ammonia analysis. The ammonia concentration was analyzed prior to the oxygen consumption test to obtain an initial ammonia concentration. Ammonia was analyzed using the phenol-hypochlorite method according to Riley et al. (1972), based on the method by Solorzano (1969). This is a colorimetric method relying on the blue color of indophenol obtained by the reaction between ammonia, phenol, and hypochlorite. Ammonium sulfate was used as the standard.

A glass vial with artificial seawater alone was used as a control for both the oxygen consumption and the ammonia excretion measurement.

Weight specific data was calculated by dividing the amount of oxygen consumed or ammonia produced by the mean individual wet weight for that stage, determined as described above.

Data analysis

The individual wet weight and length are expressed as means $\pm$ SD. All other data are expressed as means $\pm$ SEM.

An one way analysis of variance (ANOVA, Zar, 1974) was applied to the data on protein, lipid, carbohydrate, wet weight, and ash weight to determine if the variation of their values in the stages is significant. The sum of the protein, lipid, and carbohydrate percentages was compared to 100% using a Student's <u>t</u>-test. The oxygen consumption, ammonia excretion, and O:N ratio values after 24 hours of starvation were compared to the values obtained immediately after feeding using a paired <u>t</u>-test (Baily, 1959). An ANOVA was applied to compare the oxygen consumption rate, ammonia excretion rate, the O:N ratio and the specific dynamic action of the stages. The egg and nauplius V/VI stages were either excluded from the analysis of the stages, or considered as part of the starved group under the rationale that no food was offered to these stages. An one variable regression analysis (Zar, 1974) was used to determine size-metabolic rate relationship. A Student's <u>t</u>-test was applied to determine if the weight-specific exponential constants, b, in the resulting regression lines are different from one.

A significance level of 0.05 was used. At times a significance level of 0.01 is also noted in the figures.

Results

Individual wet weight and body length

The individual wet weight and body length is presented in Table III-1. The individual wet weight decreased from the egg to the nauplius V/VI and then increased with each successive stage. The total body length continually increased.

Biochemical composition

The biochemical composition of the selected stages are presented in Table III-2.

The water content decreased with each successive stage studied, from 90% in the egg to 83% in the PL 9. The water content varies significantly among the stages studied (ANOVA, $p < 0.05$).

Ash weight (% dry weight) increased with each successive stage studied, from 8% in the egg to 19% in the PL 9. These levels vary significantly.

The mean protein composition in the larval stages varied between 81% and 87% ash-free dry weight. There is no significant difference between the stages studied.

The lipid composition generally decreased with each successive stage studied, declining from 16% ash-free dry weight in the egg to 7% in the PL 9. The level varies significantly among the stages studied.

The carbohydrate composition made up the smallest fraction of the ash-free dry weight, from 1.4 to 2.8%. This percentage decreased in each successive stage tested. The variation among the stages studied is significant.

The sum of the protein, lipid, and carbohydrate fractions is not significantly different from 100% of the ash-free dry weight in the egg. In the other stages studied the sum is less than 100% (Student's t-test, $p < 0.05$). The missing fraction may be composed of chitin which was not measured in this study.

Oxygen consumption

The rate of oxygen consumption in the selected stages is presented in Figure III-2. The rate of oxygen consumption per individual increased as the egg developed to the PL 3, under both starved and fed conditions. The rate increased from a mean of 7 μg-at x 10^{-4} /individual/hour in the egg to the maximum obtained result of 1085 μg-at x 10^{-4}/individual/hour in the fed PL 3. The rate of oxygen consumption was higher in the PL 3 than in the PL 9. The rate varies significantly between stages, under both starved and fed conditions (ANOVA, $p < 0.001$).

The rate of oxygen consumption immediately after feeding is always significantly higher than that after a 24 hour starvation period (paired t-test, $p < 0.01$).

The mean specific dynamic action, as shown in Table III-3, ranged from 33, to 133% but because of the large variation between experiments these values are not significantly different from one another (ANOVA p, > 0.05).

The weight specific rate of oxygen consumption followed a similar pattern to that found per individual, but with one noticible exception. The weight specific rate in the nauplius V/VI was much greater than any other stages. The weight specific rate also varies significantly among the stages, under both starved and fed conditions (ANOVA, $p < 0.01$).

Ammonia excretion

The rates of ammonia excretion are presented in Figure III-3. The ammonia excretion per individual increased from the egg to the PL 9, from 0.15 µg-at x 10^{-4}/individual/hour in the egg to the maximum obtained value of 35.94 µg-at x 10^{-4}/individual/hour in the starved PL 9. The rate of ammonia excretion among the stages in the starved or the fed state varies significantly (ANOVA, $p < 0.01$). The mean rate of ammonia excretion appears to be lower immediately after feeding, than after a 24 hour starvation. However, this difference is only significant in the PZ III stage (paired t-test $p < 0.05$).

The weight specific rate of ammonia excretion under both starved and fed conditions remained constant in the PZ III through the PL 9 stage (ANOVA, $p > 0.05$). The weight specific rate of ammonia excretion in the N V/VI stage is significantly higher than the other stages.

Size-metabolic rate relationships

The constants a and b are presented in Table III-4. In the allometric equation representing this relationship, $M = aW^b$, M = metabolic rate (μg-at x 10^{-4}/individual/hour, and W = weight (μg wet weight). The size-metabolic rate relationship was determined for the 4 stages studied after the N V/VI. The exponential constant, b, of the regression line for oxygen consumption under both fed and starved conditions is significantly higher than zero. The b values for ammonia excretion are not significantly different from one (Student's t-test, $p > 0.05$).

The O:N ratio

The O:N ratios (Figure III-4) vary significantly among the stages for both the starved and the fed animals (ANOVA, $p < 0.05$). The O:N ratio decreased from the egg to the N V/VI. In the starved animals the O:N ratio was lower in the PZ III than in the N V/VI and then increased through the PL 3. The O:N ratio was lower in the PL 9 than in the PL 3. In fed animals the O:N ratio of the PZ III was higher than in the N V/VI and continued to increase to the PL 9 stage.

The mean O:N ratio was always lower after 24 hours of starvation than immediately after feeding. However this difference is not significant in the mysis III stage (paired t-test, $p > 0.05$).

TABLE III-1

Individual wet weight and length in selected ontogenetic stages of <u>Metapenaeus ensis</u>

Stage	Body Length (μm)	Wet Weight (μg)
Egg	288 ± 2 *	7.4 ± 2.2
Nauplius V/VI	410 ± 10	4.0 ± 2.5
Protozoea III	1854 ± 109	58.6 ± 9.4
Mysis III	3034 ± 45	135.5 ± 3.0
Postlarva 3	3190 ± 79	187.5 ± 8.3
Postlarva 9	3350 ± 35	215.0 ± 10.5

* i.e. egg diameter

Values are means ± SD

No. of determinations = 6

TABLE III-2

Biochemical composition in selected ontogenetic stages of *Metapenaeus ensis*

Stage	Water (% wet wt)	Ash (% dry wt)	Protein	Lipid	Carbohydrate
			(% ash-free dry weight)		
E	90.01 ± 0.07	7.98 ± 0.49	83.99 ± 1.43	15.90 ± 0.56	2.00 ± 0.07
N V/VI	89.43 ± 1.32	11.93 ± 1.02	81.32 ± 2.66	13.11 ± 0.95	2.22 ± 0.11 ✓
PZ III	88.25 ± 0.40	16.56 ± 0.44	85.29 ± 1.55	8.95 ± 0.96	2.19 ± 0.16 ✓
M III	85.30 ± 0.20	16.10 ± 0.59	86.16 ± 0.89	11.56 ± 0.70	1.46 ± 0.09
PL 3	84.41 ± 0.64	17.80 ± 0.87	86.46 ± 0.60	10.61 ± 0.54	1.56 ± 0.10
PL 9	83.71 ± 0.71	18.96 ± 0.92	87.13 ± 1.09	7.40 ± 0.63	1.39 ± 0.07

Values are means ± SEM

Water, n = 10

Ash, n = 8

Protein, Lipid, Carbohydrate, n = 6

n = no. of determinations

TABLE III-3

Specific dynamic action (SDA) in selected ontogenetic stages of Metapenaeus ensis

stage	% SDA (n)
Protozoea III	133 ± 44 (11)
Mysis III	59 ± 24 (10)
Postlarva 3	33 ± 7 (10)
Postlarva 9	124 ± 41 (11)

Values are means ± SEM (n = no. of experiments).

TABLE III-4

Size-metabolic rate relationship for selected ontogenetic stages (PZ III, M III, PL 3, and PL 9) of Metapenaeus ensis

		a	b	r
Oxygen consumption	Starved	0.11	1.64	0.83
	Fed	0.38	1.49	0.88
Ammonia excretion	Starved	0.17	0.96	0.73
	Fed	0.11	1.00	0.64

In the equation $M = aW^b$:

M = metabolic rate (µg-at x 10^{-4} /individual/h)

W = wet weight (µg)

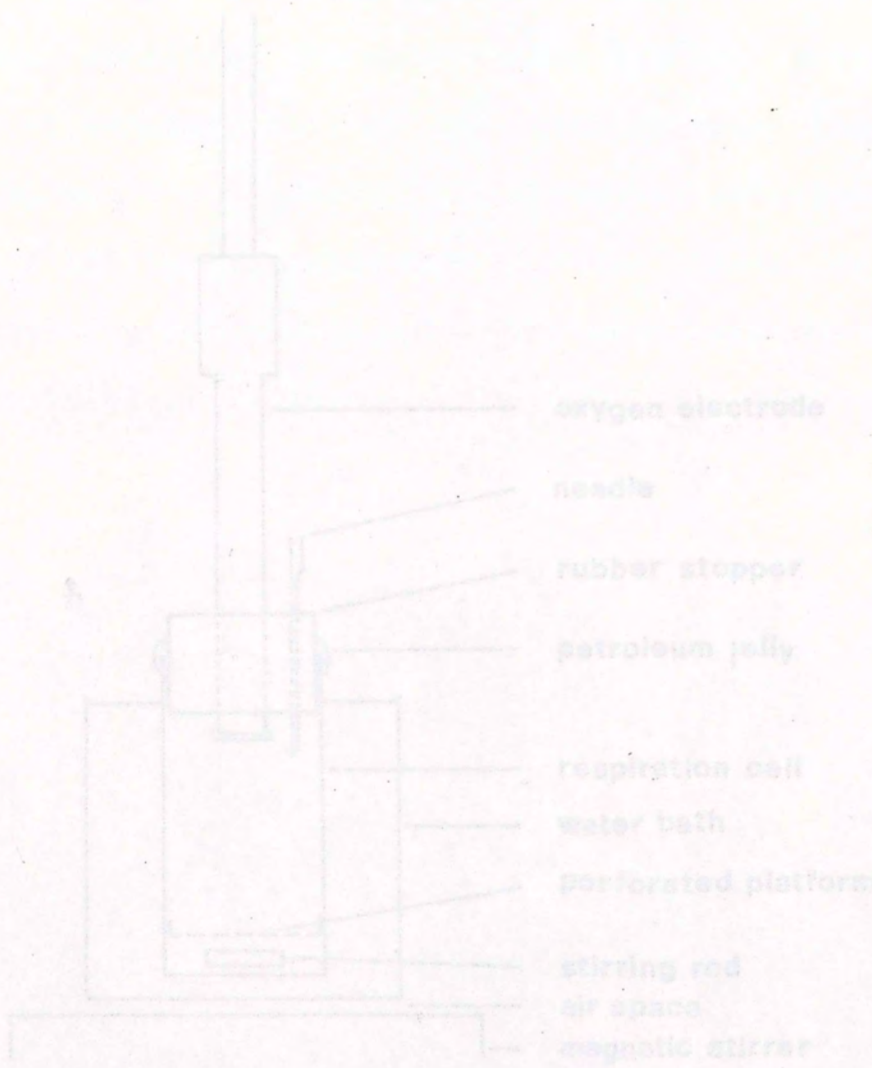
a & b = constants

r = correlation coefficient

No. of determinations = 42

FIG. III-1

Apparatus assembly for measurement of oxygen consumption in ontogenetic stages of <u>Metapenaeus ensis</u>



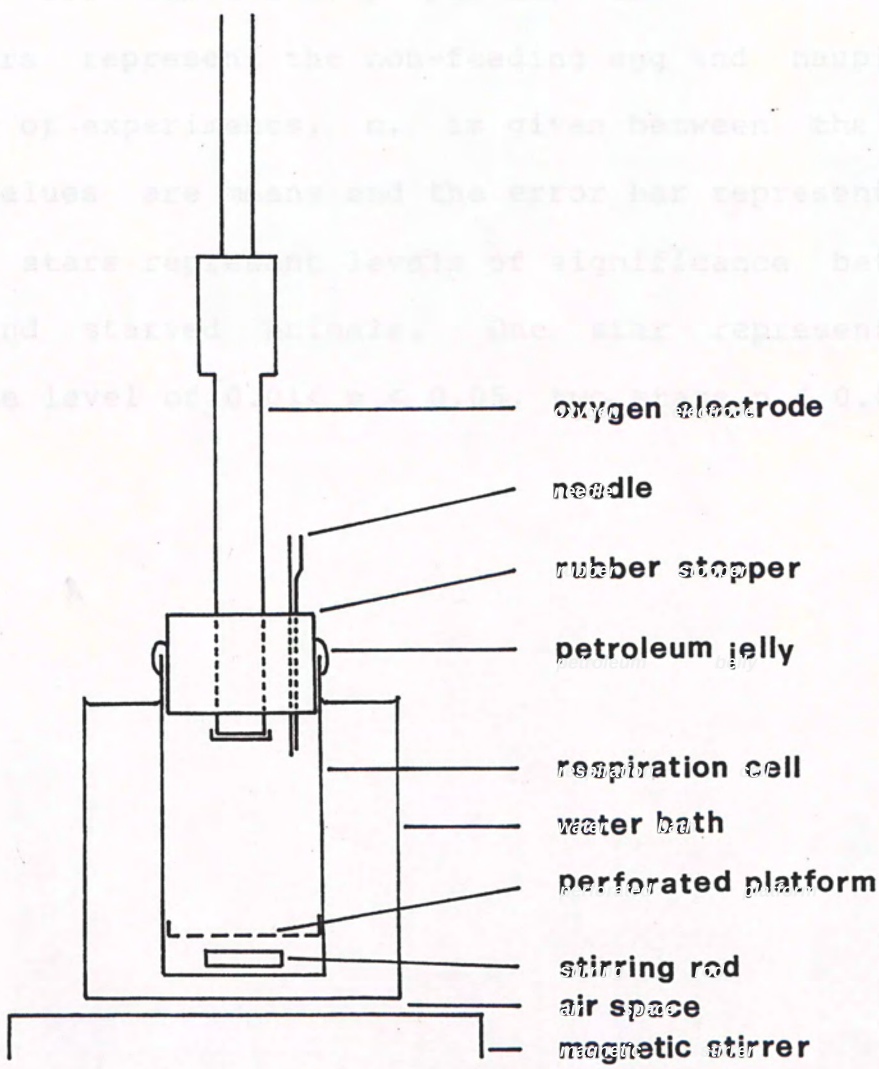

oxygen electrode
needle
rubber stopper
petroleum jelly
respiration cell
water bath
perforated platform
stirring rod
air space
magnetic stirrer

FIG. III-2.

Oxygen consumption in selected ontogenetic stages *Metapenaeus ensis*. Empty bars represent individuals that had been starved for 24 hours. Cross hatched bars represent individuals fed for a 2 hour period, after starvation. Striped bars represent the non-feeding egg and nauplius. The number of experiments, n, is given between the bar charts. Values are means and the error bar represents 1 SEM. The stars represent levels of significance between the fed and starved animals. One star represents a significance level of $0.01 < p < 0.05$, two stars $p < 0.01$.

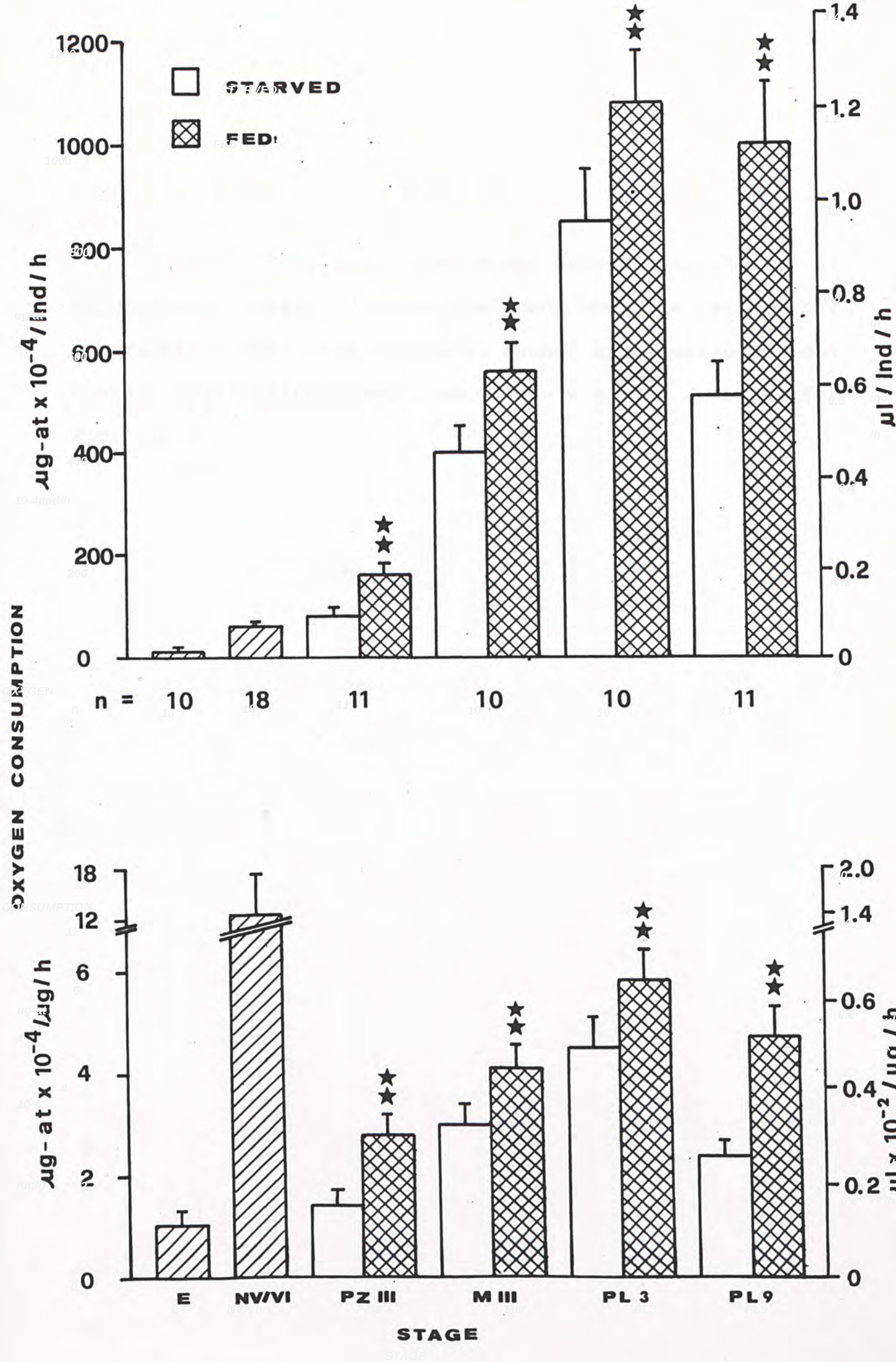

STARVED
FED
OXYGEN CONSUMPTION
µg-at x 10^-4 / Ind / h
µl / Ind / h
1200
1000
800
600
400
200
0
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
n = 10 18 11 10 10 11
µg-at x 10^-4 / µg / h
µl x 10^-2 / µg / h
18
12
6
4
2
0
2.0
1.4
0.6
0.4
0.2
0
E
NV/VI
PZ III
M III
PL 3
PL 9
STAGE

FIG. III-3.

Ammonia excretion in selected ontogenetic stages of *Metapenaeus ensis*. Values are means and the error bar represents 1 SEM. Bar hatching, number of experiments, and stars representing significance levels are as detailed for Fig. III-2.

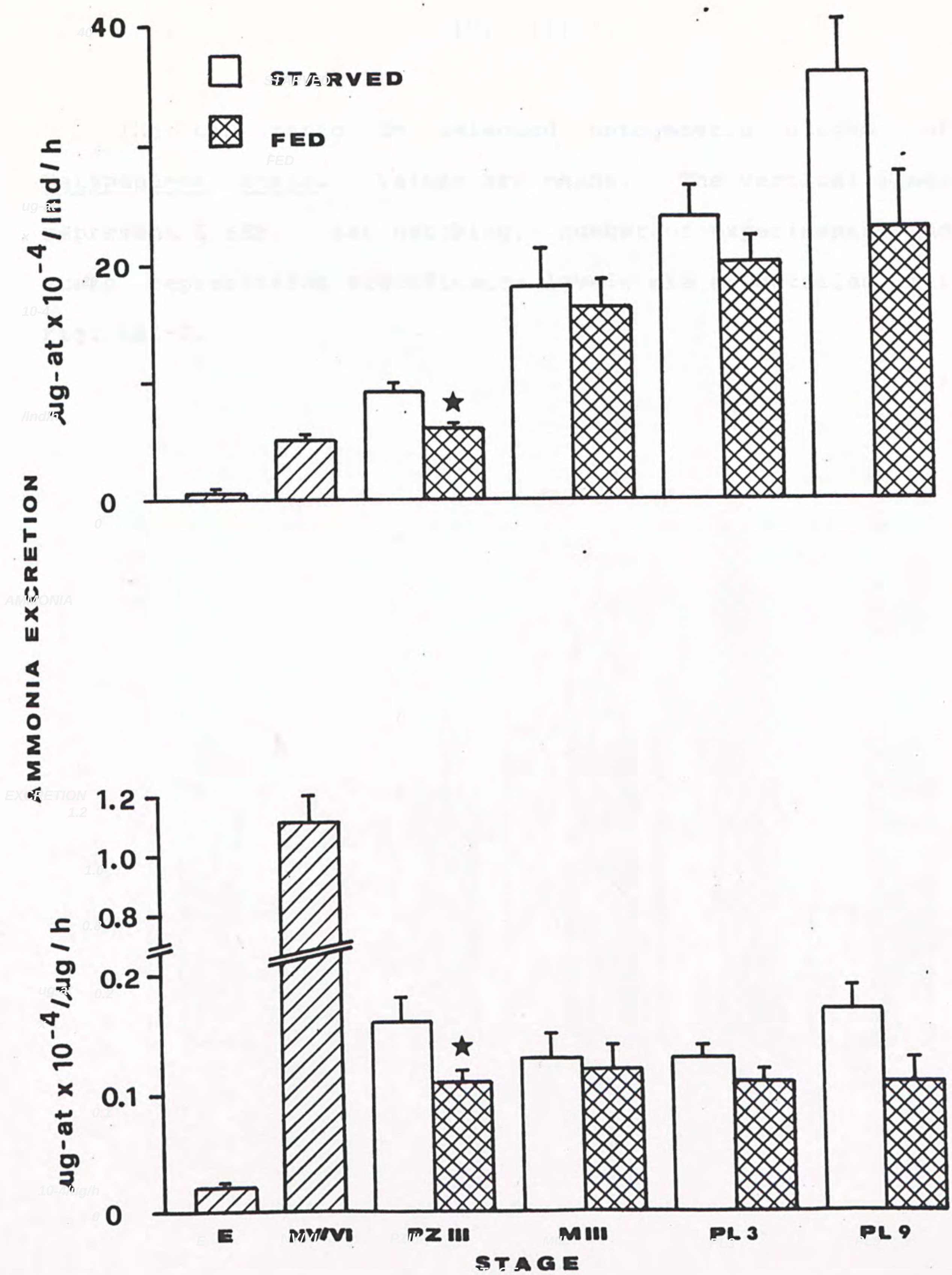
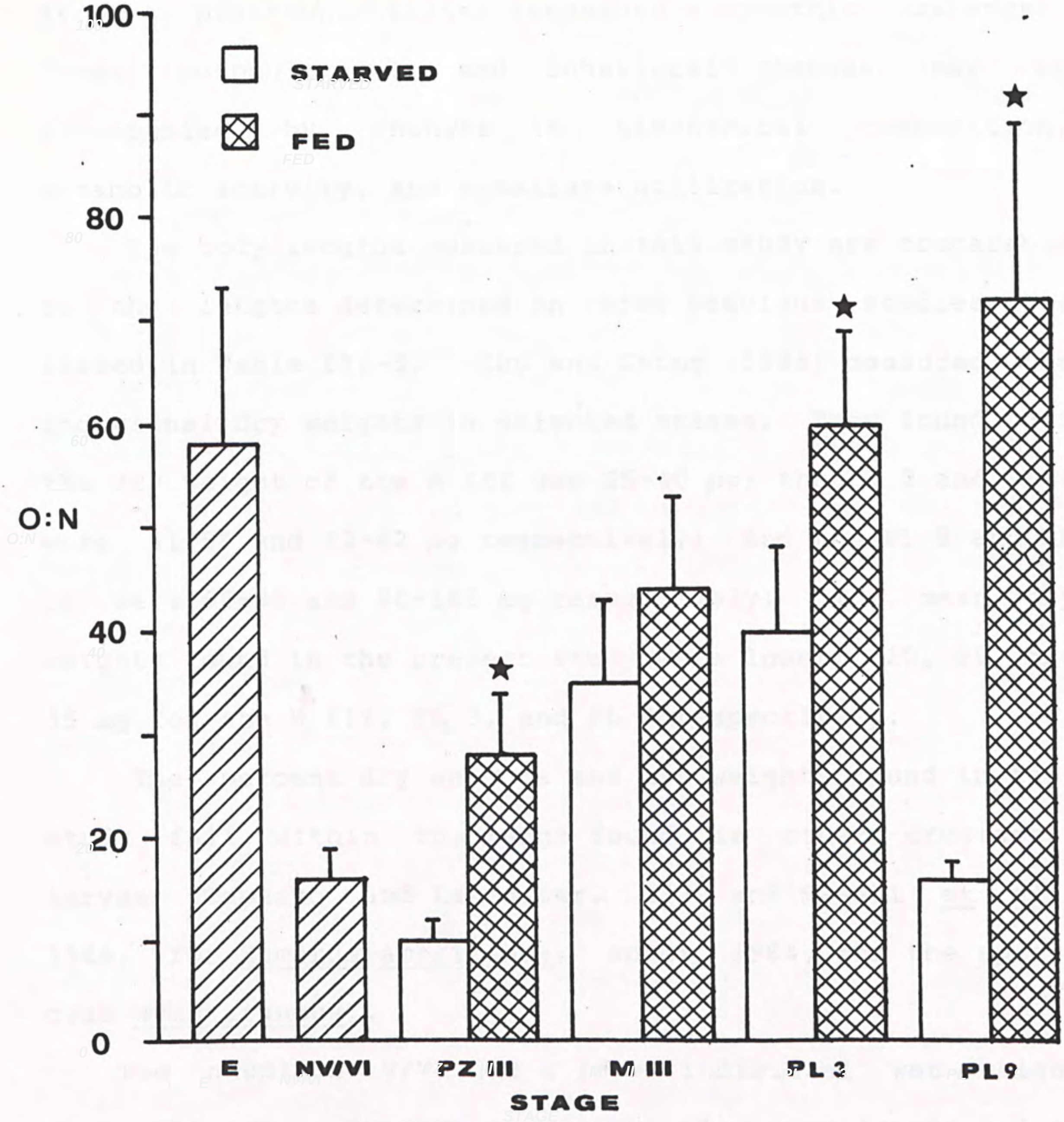

STARVED
FED
AMMONIA EXCRETION
μg-at x 10^-4 /Ind/h
μg-at x 10^-4 /μg /h
40
20
0
1.2
1.0
0.8
0.2
0.1
0
E
NV/VI
PZ III
M III
PL 3
PL 9
STAGE

FIG. III-4.

The O:N ratio in selected ontogenetic stages of <u>Metapenaeus</u> <u>ensis</u>. Values are means. The vertical lines represent 1 SEM. Bar hatching, number of experiments, and stars representing significance levels are as detailed for Fig. III-2.



100
80
60
40
20
0
O:N
STARVED
FED
E
NV/VI
PZ III
M III
PL 3
PL 9
STAGE

Discussion

During development the penaeid shrimp undergoes a sequence of changes in life style and morphology, changing from a planktonic filter feeder to a benthic scavenger. These morphological and behavioral changes may be accompanied by changes in biochemical composition, metabolic activity, and substrate utilization.

The body lengths measured in this study are comparable to the lengths determined in three previous studies, as listed in Table III-5. Chu and Shing (1986) measured the individual dry weights in selected stages. They found that the dry weight of the M III was 25-40 µg; the PL 2 and PL 4 were 41-59 and 42-62 µg respectively; and the PL 8 and PL 10 were 68-95 and 90-105 µg respectively. The mean dry weights found in the present study were lower: 20, 29, and 35 µg for the M III, PL 3, and PL 9 respectively.

The percent dry weights and ash weights found in this study fall within the range found in other crustacean larvae (Capuzzo and Lancaster, 1979 and Sasaki *et al.*, 1986, for *Homarus americanus*; Anger, 1984, for the spider crab *Hyas araneus*).

The nauplius V/VI had a lower individual wet weight than the egg. *Homarus americanus* also experiences a loss in wet weight with hatching (Sasaki *et al.*, 1986). The water content decreased with each successive stage studied, i.e. the percent dry weight increased. The ash weight also increased. Periopods and pleopods appeared as the nauplii developed to the postlarva. An increase in surface area would result in an increase in percent dry weight and ash weight. There is probably also an increasing chitin synthesis during development, which will contribute to an increase in percent dry weight. The percent chitin increases during larval development in <u>Homarus americanus</u> (Capuzzo and Lancaster, 1979). The percent dry weight and ash weight also increase in the latter <u>Homarus americanus</u> larval stages (Capuzzo and Lancaster, 1979; Sasaki <u>et al</u>., 1986).

Protein makes up the largest biochemical component in all larval and post-larval stages in <u>Metapenaeus ensis</u>. The level varied, 81-87% ash-free dry weight, but did not change with stages. This level is comparable to that found by Capuzzo and Lancaster (1979) for <u>Homarus americanus</u>, although they found a decreasing level with larval development, from 84.3 to 80.8% ash-free dry weight.

The lipid level decreased in the stages studied, from 15.9 to 7.4% ash-free dry weight (equivalent to 15-6% dry weight or 1.5-1.0% wet weight). This is somewhat higher than values of 6.8 to 4.1% ash-free dry weight found for larval <u>Homarus americanus</u> (Capuzzo and Lancaster, 1979). <u>Homarus americanus</u> also has a reduced lipid content in later larval stages.

According to Barclay <u>et al</u>. (1983), the total amount of lipid reported in the literature for decapod crustaceans has never exceeded 6% wet weig[illegible] usually much less.

Lipid levels in marine planktonic crustaceans have been reviewed (Raymont *et al.*, 1964). For the planktonic *Neomysis integer*, Raymont *et al.* (1964) reported a value of 13.1% dry weight and Linford (1965) a value of 9.5% dry weight. Conover (1968) reported values of 15-50% dry weight for *Calanus hyperboreus*, the variation being seasonal. Marine planktonic crustaceans may tend to favor a lipid based metabolism because of its higher caloric yield and buoyant character (Clifford and Brick, 1983). The higher lipid levels found in the early larval stages in the present study may therefore be related to a planktonic life style, and the reduction between PL 3 and PL 9 associated with a change to benthic existence.

Sasaki *et al.* (1986) suggested that newly settled *Homarus americanus* may rely temporarily on stored lipid reserves during the period of adaption to a new feeding behavior in the benthic habitat. The depletion of lipids from PL 3 to PL 9 in *Metapenaeus ensis* may be related to a similar phenomenon.

Carbohydrates occupied a very small percentage of the ash-free dry weight, 2.8% in the egg decreasing to 1.4% in PL 9. This is similar to the carbohydrate content of 2.5 to 1.8% ash-free dry weight in *Homarus americanus* (Capuzzo and Lancaster, 1979).

The oxygen consumption found in the present study varied between 1-12 µg-at x 10^{-4} /µg wet weight/hour (equivalent to 10-120 µl/mg dry weight/hour). In fed PL 9, the oxygen consumption was 4.6 μg-at x 10^{-4} /μg wet weight/hour (equivalent to 31 μl/mg dry weight/hour). The wet weight and dry weight of PL 9 was 215 μg and 35 μg respectively. The stage I larvae of Homarus americanus with a dry weight of 1000 μg have an oxygen consumption rate of 2.3 μl/mg dry weight/hour (Capuzzo and Lancaster, 1979). Macrobrachium olfersii larvae with a dry weight of 13-72 μg have an oxygen consumption rate of 5-7 μl/mg DW/hour (McNamara et al., 1986). The oxygen consumption reported in this study is higher. The discrepancy may be related to temperature among other factors.

The oxygen consumption on a per individual basis increased with each stage studied until the PL 3, indicating an increasing oxygen consumption with individual size. The oxygen consumption of a PL 9 was, however, less than that of a PL 3, despite an increase in size. Postlarval Metapenaeus ensis switch from being planktonic continuous feeders to a benthic existence when the postlarvae are approximately 5-6 days old (noted by Chu and Shing, 1986). A benthic animal has a lower oxygen demand than one which is more actively swimming (Dall and Smith, 1986). This reduction in oxygen consumption may also be accompanied by a change to a more nocturnal life style. After changing to a benthic existence the shrimp is no longer a continuous feeder and the nocturnal behavior may also be brought about, resulting in a decline in the oxygen consumption rate during the day time.

The nauplius had a weight specific rate of oxygen consumption approximately 100% higher than that of any other stages studied. It is expected that the nauplius had a much greater rate of oxygen consumption than the non-motile egg, but it is more difficult to explain why their rate of oxygen consumption was so much greater than in all stages studied. This difference may be partially related to molts occurring during measurement of oxygen consumption in the nauplius V/VI stage. There is usually an increased rate of oxygen consumption associated with ecdysis (Penkoff and Thurberg, 1982; Cockcroft and Wooldridge, 1985). The larvae of <u>Metapenaeus ensis</u> remain at least one day in all other molt stages so that no molting took place during oxygen consumption determination. In approximately 14 hours the nauplii pass through 6 molt stages (Chan, 1984). It is therefore impossible to determine oxygen consumption without the nauplii molting within the experimental period. The nauplii were also observed to have an extremely rapid erratic swimming behavior which would also account for their higher rate of oxygen consumption. A reduction in body weight from the egg to the nauplius, coupled with an increase in consumption rate per individual, accounts for the greater differentiation between weight specific rates than between per individual rates for the egg and nauplius V/VI.

Within a species the rate of weight specific oxygen consumption usually decreases with increasing individual size (Bertalanffy, 1957). However, this relationship may not hold true for increasing size during larval development. Changes in life style may have a stronger effect than the size on metabolic rate, due to a changing oxygen demand (Zeuthen, 1953, in Logan and Epifanio, 1978). The increase in weight specific rate of oxygen consumption between the protozoea III stage and the 3 day old postlarva, despite increasing size, indicates an increasing energy requirement with larval development through 3 day old postlarva. This increase in weight specific oxygen consumption is illustrated by an exponential constant (b) value greater than one. In the allometric equation relating metabolic activity to body size, $M=aW^b$, $b=1.64$ for the fed condition. Homarus americanus larval stages also have an increasing energy requirement with development, demonstrated by an exponential constant of 1.24 (Capuzzo & Lancaster, 1979). Anger and Jacobi (1985) found the weight specific rate of oxygen consumption to change during development in Hyas araneus larvae.

The oxygen consumption was always lower after a 24 hour starvation period than immediately after feeding. With a reduction in food, metabolic activity decreases (Klieber, 1961). The specific dynamic action (SDA) was 33-133% in this study, compared with 148% in larval and juvenile Homarus americanus (Logan and Epifanio, 1978), and 23-64% in larval Homarus americanus (Capuzzo and Lancaster, 1979). Capuzzo and Lancaster found that the SDA was greatest in the earlier more active stages. In this study, the mean SDA decreased between PZ III and PL 3. However, it was higher in the benthic PL 9. Possibly the PL 3 continues its swimming behavior under starved conditions while the starved PL 9 has a greater reduction in activity, resulting in a greater reduction in oxygen consumption. However, a switch to a nocturnal life style may be concurrent with the switch to a benthic habitat in the PL 9. Barclay _et al_. (1983) found that the decrease in respiration rate with starvation in _Penaeus esculentus_ is restricted to active metabolism. Dall and Smith (1986) found that in _Penaeus esculentus_ starvation has little effect on oxygen consumption during the daytime. The SDA in this study may not only reflect the increase in basal metabolic rate that is a result of the metabolism of products assimilated from ingested food. The increased rate of oxygen consumption was probably also a result of increased swimming activity following feeding.

The weight specific rate of ammonia excretion was in the range of 0.02-1.11 μg-at x 10^{-4} /μg wet weight/hour (0.28-15.54 μg NH_3-N /mg dry weight/hour). Values found in the literature include 1.4 μg NH_3-N/mg dry weight/hour for _Meganyctiphanes norvegica_ (Mayzaud, 1973), 0.01 μg NH_3-N/mg dry weight/hour for _Artemia salina_ (Moffet and Fisher, 1978), and 0.10-0.20 μg NH_4-N/mg dry weight/h for _Homarus americanus_ larvae (Capuzzo and Lancaster, 1979). The ammonia excretion rate reported for the N V/VI stage in this study is higher than those reported in the literature but all other rates (0.28-2.00 μg NH_3-N/mg dry weight/hour) approximate ammonia excretion rates reported in the literature.

The ammonia excretion per individual increased with each stage studied. However the weight specific rate of ammonia excretion was similar for the stages PZ III through PL 9. The ammonia excretion was very low in the egg and much higher in the nauplius V/VI stage than the other stages. As for oxygen consumption, the high rate in the nauplius may be accounted for by molts occurring during the experimental period since there may also be an increased rate of ammonia excretion associated with molting (Regnault, 1979). Unlike this study the weight specific rate of ammonia excretion in _Homarus americanus_ increases during larval development (Capuzzo and Lancaster, 1979).

There was a slight decline in ammonia excretion after feeding. The initial response to starvation is often a reduction in excretion rate (Ikeda, 1977; Borgne, 1979). With continued starvation the excretion rate may rise above the initial level, suggesting catabolism of constituent proteins (Clifford and Brick, 1979; Regnault, 1981). It is possible that with a longer starvation period the difference between ammonia excretion rate in the starved and the fed animals may be greater. After a 24 hour starvation period _Homarus americanus_ larvae have a reduced ammonia excretion rate (Capuzzo and Lancaster, 1979). This reduction may be related to a higher resistance to starvation in the larger lobster larvae. After 24 hours of starvation _Homarus americanus_ m[illegible] experiencing the effect of short term starvation with a decrease in ammonia excretion rate, while after the same time period *Metapenaeus ensis* larvae may be experiencing the effect of long term starvation with an increase in ammonia excretion. Borgne (1979) made this distinction between short and long term starvation. *Acartia clausi* experiences an initial decrease in ammonia excretion which then increases after 6 hours of starvation, although after 22 hours of starvation the level has not exceeded the original level (Mayzaud, 1973). The isopod *Porcellio spinicornis* has an increased rate of ammonia excretion after 24 hours (Bagatto, 1986).

The O:N ratio provides an indication of substrate utilization. A high O:N ratio is an indication of carbohydrate or lipid catabolism. If only carbohydrates are being catabolized the ratio approaches infinity. The O:N ratio may be used as an index of changing substrate preference during larval development. Comparison of the O:N ratio with biochemical composition may further indicate the biochemical component on which metabolism is based (Conover and Corner, 1968).

In *Metapenaeus ensis*, the O:N ratio was higher in the egg than in the nauplius V/VI stage, after which the O:N ratio, under fed conditions, increased with each stage studied. This reduction in O:N ratio from the egg to the nauplius V/VI suggests a lesser utilization of lipids or carbohydrates. The egg and the nauplius V/VI are both non-feeding stages. They must therefore be catabolizing available nutrients in the egg yolk. Biochemical analysis showed a reduction in both the lipid and carbohydrate percentage between the egg and the N V/VI stage, which also supports a greater reliance on protein catabolism in this stage. Egg yolk consists of both lipids and proteins. It is possible that the lipids are preferentially utilized in the early stage of development.

The relationship between the O:N ratio and changes in biochemical substrate cannot be as clearly shown in the other stages studied where the effect of diet comes into play. There was a further trend of reduction in lipid and carbohydrate content with development after the nauplius V/VI stage. However the O:N ratio, under fed conditions, continued to increase from 26 in the PZ III stage to 70 in the PL 9. An increasing O:N ratio reflects increasing carbohydrate or lipid catabolism. This is made even more confusing by the changing prey requirements during larval development. The protozoea III and mysis III were fed on a diet of <u>Chaetoceros gracilis</u>, a unicellular alga, while the PL 3 and PL 9 were fed on a diet of freshly hatched brine shrimp nauplii, <u>Artemia salina</u>. On a higher protein diet the O:N ratio of the 2 latter stages is still higher. The increasing O:N ratio in this study is partially a reflection of the increasing oxygen consumption rates while the ammonia excretion rates remain relatively unchanged.

In starved individuals there is a reduction in the O:N ratio, suggesting a greater reliance on protein catabolism after a 24 hour starvation. A reduction in the O:N ratio during periods of reduced feeding has also been found in Euphausia pacifica (Ikeda, 1977). Few studies have reported the variation of O:N ratio with starvation in crustacean larvae. In crustaceans which do not experience seasonal shortages of food, and therefore do not utilize large reserves, there is generally a reduction in O:N ratio with starvation (e.g. Macrobrachium rosenbergii, Clifford and Brick, 1978; Penaeus esculentus, Dall and Smith, 1986).

The PL 9 had the greatest decrease in O:N ratio after a 24 starvation period. The PL 9 also had the smallest percentage of lipid and carbohydrate reserves and must therefore rely more heavily on protein catabolism during starvation.

TABLE III-5

Comparison of individual body length of selected ontogenetic stages of *Metapenaeus ensis* measured in this study with those in three previous studies

	Present (1)	Chu & Shing (1986) (2)	Chan (1984) (3)	Ong (1969) (3)
Stage	Length (mm)			
E	0.29 ± 0.01	-	0.27 ± 0.01	-
N V/VI	0.41 ± 0.01	-	0.37 ± 0.05 (N V)	0.37 (N IV)
			0.39 ± 0.02 (N VI)	
PZ III	1.85 ± 0.11	-	2.23 ± 0.13	1.67
M III	3.03 ± 0.05	2.7-2.8	3.23 ± 0.12	3.50
PL 1	-	-	4.03 ± 0.10	
PL 2	-	3.5-3.8		
PL 3	3.19 ± 0.08	-		
PL 4	-	3.6-4.7		
PL 8	-	4.0-5.2		
PL 9	3.35 ± 0.04	-		
PL 10	-	4.0-5.4		

(1) : mean ± SEM

(2) : values estimated from graphs

(3) : mean

CHAPTER IV

Metabolic Activity and Biochemical Composition of Juvenile *Metapenaeus ensis*: The Effects of Size, Temperature, and Starvation

Introduction

Starvation affects both metabolic activity and biochemical composition of crustaceans (e.g. in the prawn *Pandalus platyceros*, Whyte *et al*., 1986). The changes in the metabolic activity during starvation may depend on the species, the length of the starvation period, and the dietary condition prior to starvation. The rate of oxygen consumption is often reduced during starvation (e.g. in the prawn *Macrobrachium rosenbergii*, Clifford and Brick, 1983; in *Penaeus esculentus*, Dall and Smith, 1986), but among different species the effect of starvation on the rate of ammonia excretion is not as uniform as its effect on oxygen consumption.

Borgne (1979) distinguished between the effects of short term and long term starvation on metabolic activity. Crustaceans frequently encounter short term starvation in the natural environment, the result of which is a reduction in the rate of oxygen consumption and ammonia excretion (e.g. in *Macrobrachium rosenbergii*, Clifford and Brick, 1979). The effect of long tèrm starvation may be a further reduction in oxygen consumption accompanied by either an increase or decrease in ammonia excretion. In addition to the length of the starvation period, the response of ammonia excretion may depend on the biochemical substrate being catabolized.

Clifford and Brick (1983) reviewed substrate utilization in selected crustaceans. In order to determine the biochemical substrate being utilized, the study of metabolic activity during starvation is often paired with that of biochemical composition analysis (e.g. in the prawn <u>Pandalus</u> <u>platyceros</u>, Whyte <u>et</u> <u>al</u>., 1986). A comparison of the O:N ratio with changes in biochemical composition over a period of starvation may suggest preferences in substrate utilization (Conover and Corner, 1968).

This study determines metabolic activity, biochemical composition, and the effect of starvation on these parameters in <u>Metapenaeus</u> <u>ensis</u>. The study is divided into three parts: 1. the determination of size-metabolic rate relationships for oxygen consumption and ammonia excretion, 2. the study of the effect of a three day starvation period and subsequent feeding on the metabolic activity, and 3. the effect of starvation and subsequent feeding on the biochemical composition.

The O:N ratios were calculated and an estimate was made on the nature of the substrate being utilized during starvation. Oxygen consumption and ammonia excretion was quantified at 2 temperatures to determine any effect temperature might have on these parameters.

Materials and Methods

Juvenile *Metapenaeus ensis* were supplied by a local shrimp farmer. They had been produced by wild caught spawners and raised in a large outdoor tank. The procedure for the larval rearing was similar to that described in Chapter III. The juveniles were placed in an aerated 500 l tank at 30 ppt salinity and 20-22 C. They were fed with a commercial dry pellet diet. The juveniles were maintained in the tank for a week before the start of experiments.

Acclimation of juveniles

Juveniles were removed from the culture tank and placed in individual beakers of artificial seawater of 30 ppt salinity, in a constant temperature water bath at either 25 or 30 C. Animals which had a soft exoskeleton and had therefore probably just molted were avoided. The juveniles were maintained in the water bath for three hours with a supply of food.

1. Size-metabolic rate relationships

The rates of oxygen consumption and ammonium excretion were determined in shrimp over a broad size range (17-177 mg). These rates were determined for animals at 2 temperatures, 25 and 30 C.

Oxygen consumption

A Gilson differential respirometer was used to determine the rate of oxygen consumption. Juveniles were removed from the constant temperature water bath and rinsed with fresh artificial seawater and placed in reaction flasks with 8 ml seawater. Potassium hydroxide solution (10%, 0.2 ml) was added to the center well for carbon dioxide absorption, with a filter paper wick to increase surface area. The system was allowed to equilibrate for at least 45 minutes before the valves were closed. The flasks were gently shaken. Micrometer readings were taken every 10 minutes for two to two and a half hours. The barometric pressure reading during the experiment was supplied by the Royal Observatory and the oxygen concentration was corrected to standard temperature and pressure conditions.

Ammonia excretion

Ammonia excretion was measured concurrently with oxygen consumption. After determining oxygen consumption a 5 ml water sample was removed from the Gilson reaction flask and analyzed for ammonia concentration as in Chapter III.

Wet, dry and ash weights

Wet weight of the juveniles was taken after determination of physiological parameters to minimize the effect of handling stress on oxygen consumption and ammonia excretion. The juveniles were then dried and ashed as described in Chapter III.

Calculations

The allometric equation relating size to metabolic rate was applied to analyze oxygen consumption and ammonia excretion. This equation is as follows (Bertalanffy, 1957):

$$M = aW^{b}$$

M = oxygen consumption or ammonia excretion/h

W = wet weight of juvenile

a and b = constants

The Q_{10} was determined by measuring oxygen consumption in 16 individuals at 25 and 30 C on two consecutive days. The formula used to calculate the Q_{10} value is (Jawed, 1969):

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{\frac{10}{T_2 - T_1}}$$

where K_1 = rate at T_1

K_2 = rate at T_2

T_1 = 25 C

T_2 = 30 C

2. Effect of starvation and subsequent feeding on metabolic activity

The effect of a 3 day starvation period on oxygen consumption and ammonia excretion was determined at 25 and 30 C. Oxygen consumption and ammonia excretion were determined as described above. Juveniles used were of a more even size range (30 - 127 mg) than in the previous study on size relationships. After determining the initial rates under fed conditions the juveniles were returned to individual beakers, without food, in a constant temperature water bath. The rates of oxygen consumption and ammonia excretion were determined each day for 3 successive days of starvation. After 3 days of starvation the juveniles were fed with a commercial pellet and metabolic activity was determined after 4 hours of feeding. The juveniles were then returned to the water bath and food was supplied before metabolic activity was determined again 24 hours after termination of starvation. The wet weight of the animal was measured at the end of the experiment.

The data for those individuals which molted during the experimental period were discarded to eliminate the effect of ecdysis on oxygen consumption and ammonia excretion rates. The experiment was performed on 4 groups of animals at each temperature and the data from the 4 groups were pooled.

3. The effect of starvation and subsequent feeding on biochemical composition

A separate group of juveniles was used for biochemical analysis. Animals were maintained in individual beakers. Samples were taken for determination of biochemical composition for each day of a 3 day starvation period and following one and two days of subsequent feeding. The juveniles were weighed every day. Animals were maintained at room temperature (approximately 25 C) during this experiment.

Wet weight and dry weight

Excess water was removed before measuring the wet weight by blotting the animal on absorbent paper. The juveniles were then freeze dried and stored at 4 C until biochemical analysis.

Protein, lipid and carbohydrate analysis

After freeze drying an individual was homogenized with water using a glass homogenizer. Aliquot samples were removed for biochemical analysis. Lipid and carbohydrate percentages were determined as described in Chapter III.

The protein percentage was determined according to Lowry *et al*. (1951). This is a colorimetric method involving the reaction of protein in an alkaline solution with cupric tartrate and the Folin-Ciocalteu reagent.

Data analysis

Data are expressed as mean ± SEM (n = number of individuals). Regression analysis was applied to the rates of oxygen consumption and ammonia excretion to determine the size-metabolic rate relationships. A Student's *t*-test was applied to determine if the slopes of the regression lines are different from zero. Oxygen consumption, ammonia excretion, and O:N ratios at 25 C were compared to those at 30 C using a Student's *t*-test. For the starvation experiment, the oxygen consumption, ammonia excretion, and O:N ratio at each successive test period was compared with the values before starvation using a paired *t*-test. The biochemical composition at each test period was compared with the values before starvation using a Student's *t*-test. A significant level of 0.05 was used except where otherwise noted on the figures.

Results

1. Size-metabolic rate relationships

The values for the constants a and b in the allometric equation $M = aW^b$, where M = rate of oxygen consumption or ammonia excretion in ng-at/h and W = wet weight of the individual in mg, are presented in Table IV-1.

A Student's *t*-test was applied to the slope of all the regression lines and they were found to be not significantly different from one ($p > 0.05$). Therefore size was found to have a negligible effect on weight specific rate of oxygen consumption and ammonia excretion within the size range of juveniles studied (17 - 177mg).

Oxygen consumption, ammonia excretion, and the O:N ratio

The rates of oxygen consumption, ammonia excretion, and the O:N ratios are presented in Table IV-2. The rate of oxygen consumption at 30 C is significantly higher than at 25 C in fed juveniles (Student's *t*-test $p < 0.05$). The ammonia excretion at 30 C is not significantly different from that at 25 C. There is no significant difference in the O:N ratio.

The Q_{10} value for oxygen consumption is 1.69 ± 0.19 ($n = 16$).

Water content and ash-weight

The water content was $80.94 \pm 0.74\%$ wet weight. The ash weight was 24.78 ± 0.94% dry weight (n = 26).

2. Effect of starvation and subsequent feeding on metabolic activity

These data are presented in Figure IV-1.

Oxygen consumption

The oxygen consumption decreased with starvation. On each day of starvation the oxygen consumption is significantly lower than the initial level (paired t-test, $p < 0.05$).

At 25 C the oxygen consumption continued to decrease over the three day starvation period. On the first, second, and third day of starvation, oxygen consumption was respectively 74, 74, and 57% of the initial level. At 30 C the oxygen consumption did not decrease further after the second day of starvation, declining with each day to 65, 52, and 54% of the initial level. The oxygen consumption appeared to have decreased more rapidly at 30 C.

Following a 4 hour feeding period the oxygen consumption rate returned to the initial level before starvation and remained at this level 20 hours later. At both temperatures the oxygen consumption at these 2 time periods is not significantly different from the initial level. After two days of starvation the oxygen consumption at the two temperatures is not significantly different from each other (Student's t-test, $p > 0.05$). At all other time periods the oxygen consumption at 30 C is significantly higher than at 25 C.

The specific dynamic action was calculated according to the following formula (Kleiber, 1961):

$$\frac{K_1 - K_2}{K_2} \times 100 = \%\ SDA$$

K_1 = oxygen consumption after the 4 h feeding period

K_2 = oxygen consumption after 3 days of starvation

The specific dynamic action at 25 C was 98 ± 15% (n = 16) and 84 ± 10% at 30 C (n = 19). The two are not significantly different from each other.

Ammonia excretion

The pattern of change in response to starvation was similar at both temperatures. There was a gradual increase in ammonia excretion to 46 and 48% above the initial level after 3 days of starvation, at 25 C and 30 C respectively. After a 4 hour feeding period the ammonia excretion was approximately 2.6 times higher than the initial level. After feeding for 24 hours the excretion rate had decreased to the initial level before starvation.

On the third day of starvation the ammonia excretion rate is significantly higher at 30 C than at 25 C. At all other time periods the ammonia excretion rates at the two temperatures are not significantly different from each other.

O:N ratios

The O:N ratio decreased with starvation at both temperatures. After the 4 hour subsequent feeding period the O:N ratio remained low but had returned to the initial level after 24 hour of feeding.

At 25 C, the O:N ratio is significantly lower than the initial value during the 3 day starvation period and after the 4 hour feeding period. At 30 C the O:N ratio is significantly lower than the initial value only after 2 days of starvation.

The O:N ratio is only significantly different between the 2 temperatures on the first day of starvation.

3. The effect of starvation and subsequent feeding on biochemical composition

The changes in biochemical composition with starvation are presented in Figure IV-2.

Wet weight

There was no detectable decline in wet weight with starvation. The precision of determining the wet weight was about $\pm$ 3 mg so that any change of less than 3-5% could not be detected. The water content did not significantly change over the 3 day starvation period (Student's t-test, $p > 0.05$).

Protein

The protein level fluctuated slightly during the three day starvation period and the two day subsequent feeding period, between 67.23 and 57.63% dry weight. This fluctuation is not statistically significant.

Lipid

The lipid level decreased with starvation. The lipid level is significantly lower on all 3 days of starvation. After feeding the lipid level returned to the initial level so that the difference from the initial level is not significant.

Carbohydrate

The carbohydrate level decreased with starvation. The carbohydrate level is significantly lower on all 3 days of starvation. After feeding the carbohydrate level returned to the initial level so that the difference from the initial level is not significant.

TABLE IV-1

Values for the constants a and b in the equation $M = aW^b$, for oxygen consumption and ammonia excretion under fed conditions in juvenile <u>Metapenaeus</u> <u>ensis</u> at 25 and 30 C

	Temp.	a	b	r	n
Oxygen consumption	25	42.82	0.91	-0.11	84
	30	95.92	0.81	-0.36	45
Ammonia excretion	25	2.18	0.77	-0.15	84
	30	3.94	0.71	-0.24	45

M = metabolic rate (ng-at/hour)

W = wet weight (mg)

r = correlation coefficient

n = no. of individuals

TABLE IV-2

Rate of oxygen consumption and ammonia excretion and the O:N ratio of juvenile Metapenaeus ensis at 25 and 30 C

Temperature C	Oxygen Consumption (ng-at/mg wet weight/hour)	Ammonia Excretion	O:N	n
25	32.6 ± 1.1	1.1 ± 0.1	51.8 ± 6.1	84
30	42.2 ± 1.6	1.3 ± 0.1	47.1 ± 5.5	45

Values are means ± SEM.

n = no. of individuals

FIG. IV-1.

Changes in oxygen consumption, ammonia excretion, and the O:N ratios with starvation , at 2 temperatures, in juvenile _Metapenaeus_ _ensis_. The dash line represents 25 C, the solid line 30 C. At 25 C values are means of 20 determinations, at 30 C of 21 determinations. Vertical lines represent 1 SEM. The arrow on each graph indicates the time when food was supplied. A single star represents a value significantly different from time = 0 at the $0.001 < p < 0.05$ level, a double star at the $p < 0.001$ level.

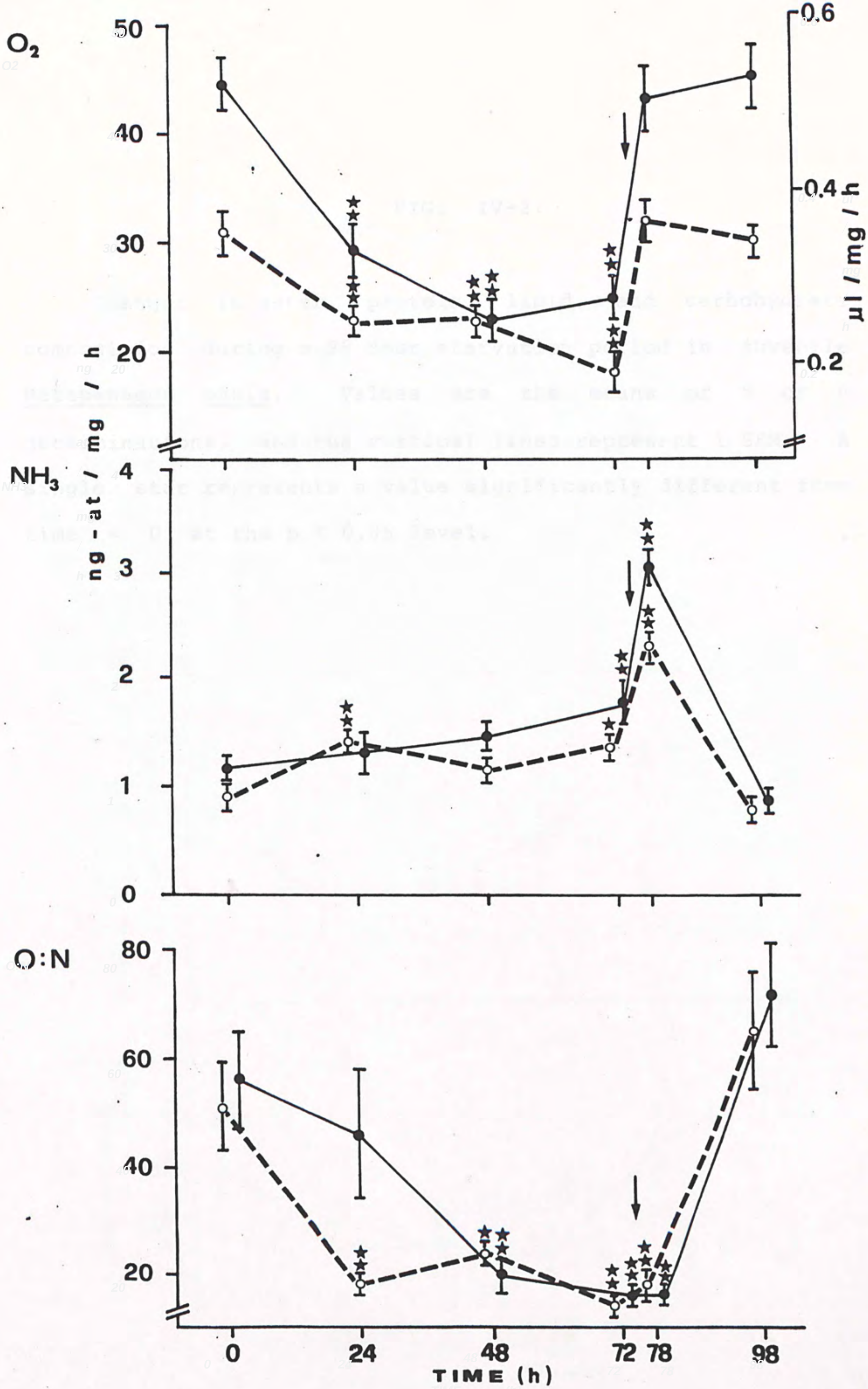
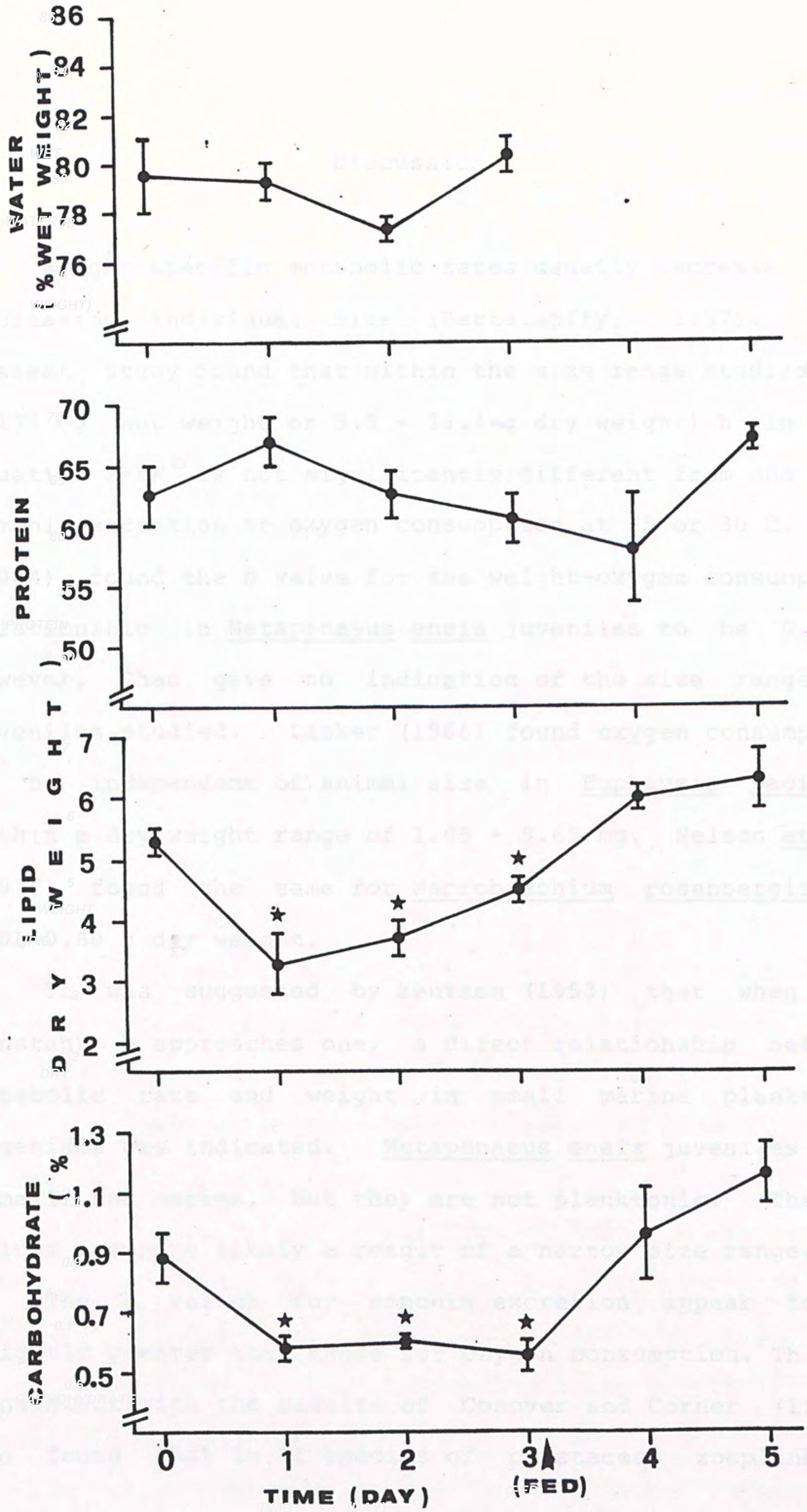

O$_2$
50
40
30
20
0.6
0.4
0.2
µl / mg / h
ng - at / mg / h
NH$_3$
4
3
2
1
0
O:N
80
60
40
20
0
24
48
72
78
98
TIME (h)

FIG. IV-2.

Changes in water, protein, lipid, and carbohydrate composition during a 96 hour starvation period in juvenile Metapenaeus ensis. Values are the means of 5 or 6 determinations, and the vertical lines represent 1 SEM. A single star represents a value significantly different from time = 0 at the $p < 0.05$ level.


WATER (% WET WEIGHT)
PROTEIN
LIPID
CARBOHYDRATE
(% DRY WEIGHT)
TIME (DAY)
(FED)

Discussion

Weight specific metabolic rates usually decrease with increasing individual size (Bertalanffy, 1957). The present study found that within the size range studied (17 - 177 mg wet weight or 3.5 - 36.4 mg dry weight) b in the equation $M=aW^b$ is not significantly different from one for ammonia excretion or oxygen consumption at 25 or 30 C. Chan (1984) found the b value for the weight-oxygen consumption relationship in Metapenaeus ensis juveniles to be 0.562. However, Chan gave no indication of the size range of juveniles studied. Lasker (1966) found oxygen consumption to be independent of animal size in Euphausia pacifica within a dry weight range of 1.05 - 9.65 mg. Nelson et al. (1977) found the same for Macrobrachium rosenbergii of 0.01-0.60 g dry weight.

It was suggested by Zeuthen (1953) that when the constant b approaches one, a direct relationship between metabolic rate and weight in small marine planktonic organisms was indicated. Metapenaeus ensis juveniles are "small" and marine, but they are not planktonic. These b values are more likely a result of a narrow size range.

The b values for ammonia excretion appear to be slightly greater than those for oxygen consumption. This is consistent with the results of Conover and Corner (1968), who found that in 11 species of crustacean zooplankton, including *Calanus hyperboreus*, *C. glacialis*, *Meganyctiphanes norvegicus*, and *Metridia longa*, the weight specific rate of ammonia excretion decreases more rapidly than oxygen consumption with increasing size.

Both oxygen consumption and ammonia excretion in *Metapenaeus ensis* were higher at 30 C than at 25 C. The effect of temperature on oxygen consumption appears to be greater than that on ammonia excretion (30 vs. 20% increase).

Between 25 and 30 C the Q_{10} value for oxygen consumption is 1.69. It is comparable to the study of Chan (1984) in which it was reported that between the temperatures of 26 and 36 C the Q_{10} values for *Metapenaeus ensis* juveniles vary between 1.57 and 1.79.

The O:N ratios calculated are 51.8 and 47.1 for 25 and 30 C respectively. A high O:N ratio is an indication of carbohydrate or lipid catabolism. The greater the proportion of protein utilized the lower the O:N ratio will be, down to a minimum of 4 - 8 when only proteins are being utilized (Conover and Corner, 1968; Mayzaud, 1973). These high O:N ratios indicate that primarily a non-protein source is being utilized.

The biochemical composition, before starvation, reported in this study is similar to that reported for *Neomysis integer* (Raymont et al., 1964), *Macrobrachium rosenbergii* (Choo, 1973), and postlarval *Homarus americanus* (Capuzzo and Lancaster, 1979).

In the absence of feeding, the oxygen consumption decreased. In crustaceans there are basically two patterns of response of oxygen consumption to starvation documented in the literature. The response of oxygen consumption to starvation may be characterized by a high initial decrease followed by a leveling, similar to that found in this study for Metapenaeus ensis, or by a small initial decrease followed by a lag period before a greater decrease in respiration rate (e.g. in the shrimp Crangon crangon, Regnault, 1981). After a 24 hour starvation period the respiration rate in Metapenaeus ensis juveniles had already decreased sharply. Dall and Smith (1986) found that in Penaeus esculentus of about 20 g wet weight, respiration rate decreases rapidly with starvation to 76-71% of the initial level after 5 days. Kulkarni and Joshi (1980) found that Penaeus japonicus follow a similar pattern, decreasing respiration rate to about 85% of the initial level after 4 days of starvation (animals size within 4-15 g). Greater decreases, closer to those found in the present study, have been reported for smaller organisims. Anger (1986) reported for Hyas araneus larvae (64 - 240 µg dry weight) a decrease of about 50% the initial level after 4 days of starvation.

Ammonia excretion increased with starvation in Metapenaeus ensis. The increase in ammonia excretion was gradual and did not level off within the experimental period. Increases in rate of ammonia excretion with starvation in crustaceans has also been demonstrated in

Calanus hyperboreus (Conover and Corner, 1968), in *Penaeus esculentus* (Dall and Smith, 1986), and in *Carcinides maenas* (Needham, 1957).

Borgne (1979) distinguished between short term and long term starvation. The result of short term starvation is usually a small decrease in both respiration and nitrogen excretion rates. This pattern was demonstrated by Regnault (1981) who found an initial decrease in ammonia excretion followed by an increase in *Crangon crangon*. Ammonia excretion rates in *Metapenaeus ensis* juveniles would have to be measured after starvation time periods of less than 24 hours to determine if an initial reduction in ammonia excretion was experienced. The fluctuation in ammonia excretion rates with starvation is linked to changes in substrate catabolism. The O:N ratio may be utilized as an indication of substrate utilization. The O:N ratio is at best a qualitative index (Clifford and Brick, 1983). Comparison of the O:N ratio with changes in biochemical composition will more accurately suggest substrate utilization during starvation.

The O:N ratio of *Metapenaeus ensis* juveniles decreased with starvation. A decrease in the O:N ratio suggests that the juvenile under starved conditions was relying more heavily on a protein rather than a carbohydrate or lipid source for catabolism. The decrease in the O:N ratio was not so great as to suggest a total reliance on proteins but rather a mixture of substrates.

Protein is the main energy source during starvation in the Norwegian lobster Nephrops norvegicus (Dall, 1981) and Penaeus esculentus (Barclay et al., 1983). The prawn Pandalus platyceros relies primarily on lipid reserves during starvation (Whyte et al., 1986). Clifford and Brick (1983) found that in Macrobrachium rosenbergii carbohydrate was utilized preferentially, lipid and protein were secondary and tertiary substrates respectively. However, if the period of starvation was extended the role of carbohydrates was reduced. Carbohydrate reserves are also quickly depleted by Crangon crangon and lipid and protein are the main substrates oxidized during starvation (Regnault, 1981). Substrate utilization during starvation varies between crustaceans and may depend on environmental factors and dietary condition before starvation.

In the present study, the carbohydrate and the lipid fractions in Metapenaeus ensis both declined after the first day of starvation. This immediate decrease suggests that both carbohydrates and lipids were preferentially catabolized as an energy source at the onset of starvation. The O:N ratio for a period of starvation less than 24 hours may be increased, illustrating constituent carbohydrate or lipid utilization. After 24 hours of starvation the O:N ratio had already decreased. Although constituent carbohydrates and lipids may be preferentially catabolized the amount available may be insufficient and constituent proteins were catabolized as well, resulting in an increase in ammonia excretion and a decrease in the O:N ratio.

Although there was a decrease in the C:N ratio with starvation which suggests constituent protein catabolism, there was no decrease in the protein percentage. It is possible that within a 3 day starvation period, although the rate of ammonia excretion might increase, indicating constituent protein utilization, the amount of protein catabolized was too little to result in a significant loss of protein.

The total amount of protein which a fasting juvenile may utilize within a 3 day period can be calculated from the oxygen consumption. One mole of oxygen can convert 22 g of protein to ammonia (Gnaiger, 1983). Assuming that the oxygen consumption rate was uniform within a 24 hour period, in a 3 day starvation period a juvenile of 10 mg dry weight consumed approximately 10 µmoles of oxygen, which would catabolize 220 µg of protein. Protein made up 63% of dry-weight. Therefore, in a juvenile of 10 mg dry-weight, 220 µg of protein was equivalent to 3.5% of the total protein content. In other words, if a juvenile was relying solely on proteins, the protein fraction would have decreased by 3.5% in a 3 day period. Thus, even if protein was the sole substrate utilized, a 3% decrease in the protein fraction can hardly be measured.

Weight equivalents of oxygen consumption may also be calculated for carbohydrates and lipids. If in a 24 hour period the juvenile was utilizing only carbohydrates, 100% of the initial fraction would have been catabolized. Sole reliance on lipids would result in 75% of the initial lipid fraction being catabolized. From these calculations, it is likely that although carbohydrates and lipids may be preferentially catabolized at the onset of starvation, a mixture of substrates was being utilized soon after, as suggested by the O:N ratios determined. The replenishment of the carbohydrate and lipid fractions after subsequent feeding confirms that they were depleted as a result of starvation. The carbohydrate fraction in Metapenaeus ensis was about 1% dry-weight, it is unlikely that this fraction could play a large role during prolonged starvation.

Mayzaud (1973) suggested 3 metabolic levels of resistance to starvation in planktonic crustaceans. This theory may be applied to Metapenaeus ensis. Possibly during starvation there is an initial carbohydrate-lipid-protein based metabolism; secondly an intermediate level represented by a decrease in the O:N ratio; and finally severe protein loss, at which point the O:N ratio will decline to the minimum. After 3 days of starvation Metapenaeus ensis juveniles are probably between the first and second stages.

The oxygen consumption rate returned to the initial level within 4 hours of feeding yet the ammonia excretion rate did not immediately return to the pre-starvation level. Wieser (1972), working on the isopods Porcellio scaber and P. pictus, and Ganf and Blazka (1974), working on the cyclopoid Thermocyclops hyalinus, suggested that although the calorigenic effect of feeding is immediately reflected by increased oxygen consumption, there is a lag time involved for ammonia excretion to return to the pre-starvation level. Another simpler explanation may be that the oxygen consumption increased immediately due to increased activity after feeding. The juvenile shrimp was contained in a small reaction flask, in a volume of 8 ml, but it was observed that it was possible for it to move around.

Although the ammonia excretion had not returned to the pre-starvation level after 4 hours of feeding, there was a great increase in ammonia excretion, to a level higher than the initial level. The surge in ammonia excretion may be due to superfluous feeding. According to Blazka et al. (1982), in Daphnia hyalina and the copepod Cyclops vicinus, animals at low natural feeding levels exhibit the lowest rate of ammonia excretion. In animals that are superfluously feeding, the ammonia excretion increases because a sufficient amount of protein is being consumed for both production and catabolism. The relationship between assimilation efficiency and feeding level was reviewed by Grahame (1983), who stated that copepods adapt to superfluous feeding with high assimilation efficiencies, but only after an initial decline when superfluous feeding follows a period of low food intake. In this study, 4 hours after the resumption of feeding animals may be utilizing protein for both catabolism and production, resulting in a high ammonia excretion rate.

The effect of a 5 C temperature variation on the response of metabolic activity to starvation appears to be negligible. Perhaps a slightly larger temperature difference is required to clearly illustrate any effect of temperature on the above response.

CHAPTER V

General Conclusions

This study quantified oxygen consumption, ammonia excretion, and the biochemical composition of Metapenaeus ensis larvae, postlarvae, and juveniles. The O:N ratios were calculated from these data. The effect of starvation on metabolic activity in the larval and postlarval stages was studied as well as the effect of starvation on metabolic activity and biochemical composition in juveniles. The effect of a 5 C temperature difference on metabolic activity was determined in the juveniles.

The summary of the reported results and major conclusions which can be drawn from these data follows:

1. The O:N ratio declined between the egg and the N V/VI. This is coupled with a decline of lipid and carbohydrate levels indicating a greater utilization of protein, possibly the protein fraction of the egg yolk, in the nauplius.

2. During larval development the lipid and carbohydrate fractions both decreased indicating utilization of these reserves and a decreasing importance of these fractions as an energy reserve. Lipids have a high

buoyancy, so that the reduction in lipids may also be related to the switch from a planktonic to a benthic existence at about PL 5. The size of the lipid and carbohydrate fractions in the juvenile were not different from those in the PL 9. The protein fraction remained constant in all stages studied.

3. The water content decreased during development and was lower in the juveniles than in the PL 9, whereas the ash level increased with development and was higher in the juvenile than in the PL 9. Both trends may be related to development of pleopods and periopods in the larval stages. The increased dry weight may also be related to an increased chitin content.

4. Between the PZ III larval stage and the PL 3 an increasing rate of oxygen consumption indicated an increasing energy demand. There was a reduction in oxygen consumption in the PL 9 associated with the shift to a benthic existence.

5. The O:N ratio increased between the N V/VI stage and the PL 9 (in the animals that had been fed) and was the same in the juvenile as the PL 9. This indicates a greater utilization of dietary carbohydrates or lipids in the later developmental stages.

6. In the larval and postlarval stages, the effect of a 24

hour starvation period was a reduction in oxygen consumption, but the ammonia excretion was little effected. The O:N ratio after a 24 hour starvation was lower.

7. Size was found to have a negligible effect on metabolic rate within the size range of juveniles studied. However, the larvae did have a much higher metabolic activity than the juveniles. At 25 C the PL 9 (0.22 mg wet weight) had a weight specific oxygen consumption rate of 465 ± 57 ng-at/mg/hour. Juveniles (average weight 67 mg) had an oxygen consumption rate of 33 ± 1 ng-at/mg/hour. The difference in ammonia excretion rates was not as great. The PL 9 had an ammonia excretion rate of 11 ± 2 ng-at/mg/hour. For juveniles the rate was 1.1 ± 0.1 ng-at /mg/hour. The reduction in metabolic activity is related to a reduced weight specific metabolic activity with an increase in animal size and possibly to reduced activity in the juveniles.

8. In the juveniles the carbohydrate and lipid fractions declined after a one day starvation period. This decrease, coupled with the continued increase in ammonia excretion over a 3 day starvation period and a decreased O:N ratio, suggests initial utilization of carbohydrates and lipids, followed by a carbohydrate-lipid-protein mixture.

This study investigated physiological changes during crustacean larval development. Several previous studies have quantified physiological aspects in crustacean larvae. The species studied include the American lobster, *Homarus americanus* (Logan and Epifanio, 1978), *Macrobrachium rosenbergii* (Stephenson & Knight, 1980), and the spider crabs *Hyas araneus* (Anger and Jacobi, 1985; Jacobi and Anger 1985a) and *H. coarctatus* (Jacobi and Anger, 1985a, b). Studies which have linked metabolic activity with changes in biochemical composition, in an attempt to suggest substrate utilization as in this research, include those by Capuzzo and Lancaster (1979) and Sasaki *et al.* (1986), working on *Homarus americanus* larvae, and by Whyte *et al.* (1986), working on the spot prawn *Pandalus platyceros*. This study is the first of this kind to examine the larval stages of a penaeid shrimp, which has the most complicated life history among crustaceans, experiencing a complex series of changes in dietary requirements during their larval and postlarval development.

This study investigated changes in metabolic activity and biochemical composition and suggested correlations between metabolic and behavioral changes during development. It was determined that in *Metapenaeus ensis* the biochemical composition and the O:N ratio both change during development. An understanding of changes in metabolic activity and energy utilization is useful in the development of successful mariculture hatchery techniques. In order to achieve optimal diet utilization, it is important to change the feeding regime to accommodate the changing dietary requirements. Changes in substrate utilization, as suggested by the O:N ratio, indicate these changing dietary requirements. Expansion of the present study to include other larval stages might be necessary to pinpoint changes in dietary requirements.

Physiological studies can help to confirm changes in dietary requirements suggested by morphological and behavioral changes. Metabolic activity, however, is affected by a wide range of parameters, and therefore conclusions made from results of physiological studies may be somewhat speculative. Evaluating experimental diet by determining metabolic activity and biochemical composition, as in the studies on Macrobrachium rosenbergii (Clifford and Brick, 1978, 1979) and Homarus americanus (Capuzzo, 1981), will yield a more complete picture of dietary requirements. The present study can act as an initiative for future research on comparison of the effects of diet and dietary levels on metabolic activity in the penaeid shrimp.

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